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Mode of Penetration and of Progressive Invasion of Fire-Blight Bacteria Into Apple and Pear Blossoms

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IMPERIAL MYCOLOGICAL INSTITUTE
18 MAR. 1937

BULLETIN NO. 331

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FAYETTEVILLE, ARKANSAS
June, 1936

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MODE OF PENETRATION AND OF PROGRESSIVE INVASION OF FIRE-BLIGHT BACTERIA INTO APPLE AND PEAR BLOSSOMS

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For many years following the pioneer work of Waite *(17, 18, 20)*, it has been assumed that penetration of apple and pear blossoms by fire-blight bacteria, *Erwinia amylovora*, is simply a process involving the passage of bacteria through a glandular surface lacking a cuticle, occurring in the receptacle cup of the blossom. The particular tissue involved in this penetration is often referred to as the nectarial region because it is the tissue which exudes floral nectar, and the main reason assigned to the ease of bacterial penetration in this tissue is the presumed absence of a cuticle.

Within recent years, the question of floral penetration by *Er. amylovora* has been reopened by a number of investigators, and because of its interest, not only from a scientific viewpoint but from its possible relationship to control measures against fire blight, particularly blossom blight, it is one of outstanding importance.

Review of Literature

Following Waite's work, previously cited, which was performed in the early nineties of the last century, there does not appear to have been any significant additional investigations of floral penetration by the fire-blight pathogen until 1929. It is true that in 1913 Bachmann *(1)* made a valuable contribution in calling attention to the passage of fire-blight bacteria through intercellular spaces, but dismissed the whole question as to the manner in which the bacteria gain entrance into pear or apple blossoms in the following words: "As is well known, it is not necessary to make any mechanical injury to secure infection, for the bacteria readily gain entrance into the tissues through the nectary." She inoculated the nectarial surface, or "outer calyx cup," of pear blossoms with bouillon cultures of *Er. amylovora* and succeeded in obtaining infections. How the bacteria entered the "nectary" was completely ignored.

In 1929 there appeared in close order the work of Miller *(7)* in Wisconsin, and Rosen *(11)* in Arkansas dealing, among other things, with this question. Miller sprayed pure cultures of bacteria on unopened blossom buds of apple and pear and obtained fire-blight symptoms within 3 to 6 days. He noted brownish discolorations on the tips of exposed sepals and apices of leaves

surrounding the blossom buds, with bacterial exudate issuing from the discolored parts, and was able to isolate the pathogen from such artificial infections. Likewise, in order to prove that the bacteria are capable of penetrating the floral tissues through natural openings, without intervention of wounds, he removed the stamens and styles of open apple blossoms, filled the receptacle cups with cocoa butter so as to cover the nectaries and wounds made by removing the stamens and styles, sprayed pure cultures of *Er. amylovora* upon sepals and outer receptacle walls, and obtained fire-blight infections on the sides of the fruit within 5 to 10 days following inoculation. He obtained similar results on unopened apple blossoms inoculated in the field and observed stomatal penetration of sepals.

Miller's studies of nectarial penetration of apple and pear blossoms are of special interest. Stained preparations of artificially infected blossoms showed the bacteria "gaining access to the host tissues through openings in the receptacle cups which appear to be stomata. In none of the flowers sectioned . . . have the bacteria been found gaining access to the tissues of open flowers except by entering stomata. The writer has been unable to find evidence in the literature (see Eames and MacDaniels p. 78) or in his own preparations that the floral nectaries of apple and pear offer an open avenue for the entry of bacteria, as no intercellular spaces which pass from the surface through this tightly compacted layer have been demonstrated." No notice is taken of the presence or absence of cuticle. It will be revealed later that Miller's supposed failure to observe nectarial penetration is probably due to the erroneous interpretations of the constitution of nectarial tissue, and it will also be shown that his finding of stomatal penetration on the nectarial disk concerned itself in part, at least, with nectarial penetration.

The writer's earliest published account (1929) of floral penetration *(11)* recorded the artificial production of disease by spraying pure cultures of *Er. amylovora* on pear flower buds, and noting infections appearing on peduncles, outer walls of receptacles, on calyx lobes, and on petals. Likewise, histological studies were presented of petal infections. Following this, further studies were presented in 1933 *(12)* involving additional data on artificial inoculations and disease production on pear blossoms in the absence of wounds or insects.

In 1931 Pierstorff *(9)*, among other studies, presented the results of his observations on the mode of entrance of fire-blight bacteria into blossoms. He produced artificial infections on petals, which had been produced previously by another investigator *(11)*, and made the first contribution to our knowledge of stigmatic infections, producing the disease by brushing the stigmas with pure

cultures of the organism. He concluded, however, that stigmatic infections as well as petal infections are not likely to be common under natural conditions. By atomizing apple blossoms with bouillon cultures of the organism and examining killed and stained preparations, he concluded that the nectarial region appears to be the point most frequently attacked, and he assumes that entrance into the tissues in this region is accomplished by rapid multiplication of the bacteria in the excreted nectar, which results in the production of enzymatic or toxic secretions. Nothing is said about the presence of any natural openings in the nectarial region and his statement, illustrated by photomicrographs, that the bacteria attack the base of the style and progress rapidly toward the center of the receptacle, leaves grave doubts whether he actually obtained or observed nectarial penetration. So far as the present writer knows, no investigator has considered the basal portion of the style as capable of secreting nectar, or as being part of the nectarial tissue.

In 1935 the writer *(13)* published a brief preliminary account of the mode of penetration of pear and apple blossoms by the fire-blight pathogen, calling attention to the following: There is a well-defined cuticle covering the nectarial region of both pear and apple blossoms. The nectar, instead of exuding from naked cells, as commonly assumed, passes out through stoma-like openings, the openings seemingly being regulated by guard cells as in true stomata. For these nectar-exuding structures the term, "nectarthodes," was proposed. In the nectarial region of pear and apple blossoms, the fire-blight pathogen gains entrance into the interior by means of these nectarthodes though entrance through these is apparently not nearly as common on apple blossoms as on pear. The reason for this difference was ascribed to the narrow, elongated, tightly-covered calyx cup, characteristic of apple blossoms during nectar flow, contrasted with the broad, open, and shallow calyx cup which is characteristic of pear blossoms. In addition to penetration through nectarthodes, *Er. amylovora* was noted as penetrating with ease the stigmatic surfaces of pear and apple gynoecia, the large glandular naked cells of these surfaces making penetration a relatively simple matter, and also penetrating the locules of the anthers.

Almost simultaneously with the appearance of the writer's report on floral penetration, there appeared an abstract of a paper presented by Hildebrand and MacDaniels *(4)* before the mid-winter meetings of the American Phytopathological Society, presenting essentially the same findings.

The report which follows presents the evidence for the statements given in the brief preliminary account, and also presents numerous significant details not previously reported.

Histological Studies of Penetration and Progressive Invasion of Pear Blossoms by Er. amylovora

In order to gain a clear understanding of the mode of entrance of fire-blight bacteria into pear blossoms, it may be desirable to call attention to a few structures present in normal pear blossoms that play an important part in acting as avenues of infection.

As artificial infections with pure cultures of fire-blight bacteria, as well as studies of natural infections, indicate that on pear blossoms the nectarial region constitutes one of the most common tissues, and often the only one, through which the bac-

Figure 1. Normal Kieffer pear blossoms. Note the open, fully-exposed nectarial region between the styles and stamens, and compare with Figure 2.

Figure 2. Normal apple blossoms slightly enlarged. Note that the nectarial region is completely covered by the combined androecium and gynecium. Compare with Figure 1.

teria gain entrance into blossoms, a careful histological study has been made of the nectarial tissue. Unlike apple blossoms (Figure 2[1]), the nectarial region of pear blossoms of commonly grown varieties takes the form of a shallow, open, and fully exposed greenish, saucer-like tissue (Figure 1) which measures approximately 5 mm. in diameter when the blossom is fully expanded. The peripheral region of this shallow saucer is marked by the stamens of which there are 20, with some variations from that number. The center of the saucer is marked by a rather deep pit through which the five styles emerge. It is the tissue between the styles and the stamens which is the nectar-bearing

[1]All photographs and drawings made by the writer.

area, as may be readily observed in pear blossoms during nectar flow by examining them under a low-powered binocular microscope. As the blossoms age and shortly after petal fall, the five sepal lobes, which are at first reflexed and curved downward in young open blossoms, shortly commence to curve upward and gradually form a somewhat deep calyx cup in the young pear fruit. Simultaneous with the upward movement of the calyx lobes, the margin of the nectarial tissue assumes a more vertical position resulting in a marked contraction of the diameter of the nectar-bearing surface. At this stage, or shortly after, depending presumably in part on weather conditions and on rapidity of fertilization, the nectar usually ceases to flow. As a whole, the writer's observations suggest that during cool, moist weather, or when twigs bearing blossom spurs are severed from trees and placed in water, the period of nectar flow is more prolonged.

Since blossom nectar by itself forms an excellent medium for the growth of fire-blight bacteria, according to Waite *(17)*, to Gossard and Walton *(3)*, and as confirmed by the writer's observations, it is obvious that the longer the interval of time involving such excretion, the greater the chance for bacterial invasion of this nectar and, ultimately, of the blossom. However, other factors than the length of time involved in nectar excretion doubtless have much to do with bacterial growth and invasion of blossoms, and Thomas and Ark *(16)* have called attention to the variation in concentration of sugars found in pear and other nectars which may materially influence the growth of *Er. amylovora.*

As observed under a low-powered binocular microscope, the nectar may be seen in small discrete droplets resting in areas which are often lower than the main surface, and gathered in pits or depressions, though this is not always the case. If the droplet is carefully brushed aside, other droplets may at times be seen issuing into the same pits. These droplets can more or less readily be seen to flow out of stoma-like structures. When the flow of nectar is comparatively large, the droplets join and their points of origin from the tissue are not easily determined. However, there is usually no difficulty in observing the flow through the stoma-like openings when the blossoms are exuding nectar if the bases of the blossom spurs are kept immersed in water until they are mounted under the microscope.

Histological studies of the nectar-secreting tissue reveal the following: In surface view, easily observed by floating the nectarial tissue in Amann's mounting medium on a glass slide and heating for a few minutes, the epidermal cells appear as relatively small, often rectangular-shaped structures, between which there commonly appear what seem to be stomata, interspersed throughout the epidermal layer. These consist, in surface view, of two curved, sausage-shaped guard cells, each of which is usu-

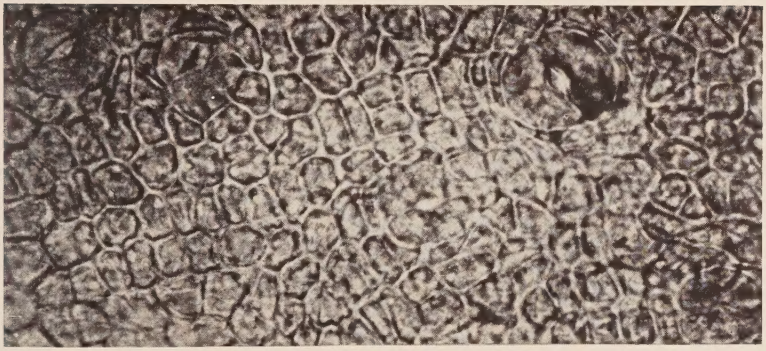


Figure 3. (Above.) Surface view of nectarial region of Kieffer pear blossoms showing the openings made by the nectarthodes through which the nectar is secreted. Magnified 586 ×.

Figure 4. (Below.) Longitudinal section through a Bartlett pear receptacle in the nectarial region showing cuticle and nectarthodes. Magnified 600 ×.

ally several times as large as the surrounding epidermal cells (Figure 3), and between which can be seen a stoma-like opening. The opening varies in size as in normal stomata.

Longitudinal sections through the nectarial tissue, either of fixed, paraffin embedded, microtome sectioned, and stained preparations, or of free-hand sections, show a well-defined cuticle covering the whole region other than the openings between the guard cells (Figures 4, 5, and 6). This cuticular covering is fully as thick as that lining the epidermal cells of the outer walls of the

Figure 5. (Above.) Longitudinal section through a normal Kieffer pear receptacle in the nectarial region showing cuticle and the characteristic imbedded type of nectarthode. Magnified 586 ×.

Figure 6. (Below.) Longitudinal section through a normal Bartlett receptacle in the nectarial region, showing the opening made by the guard cells and the heavily-stained material, probably representing nectar, reaching from the sub-nectarthodal chamber to the surface. Magnified 950 ×.

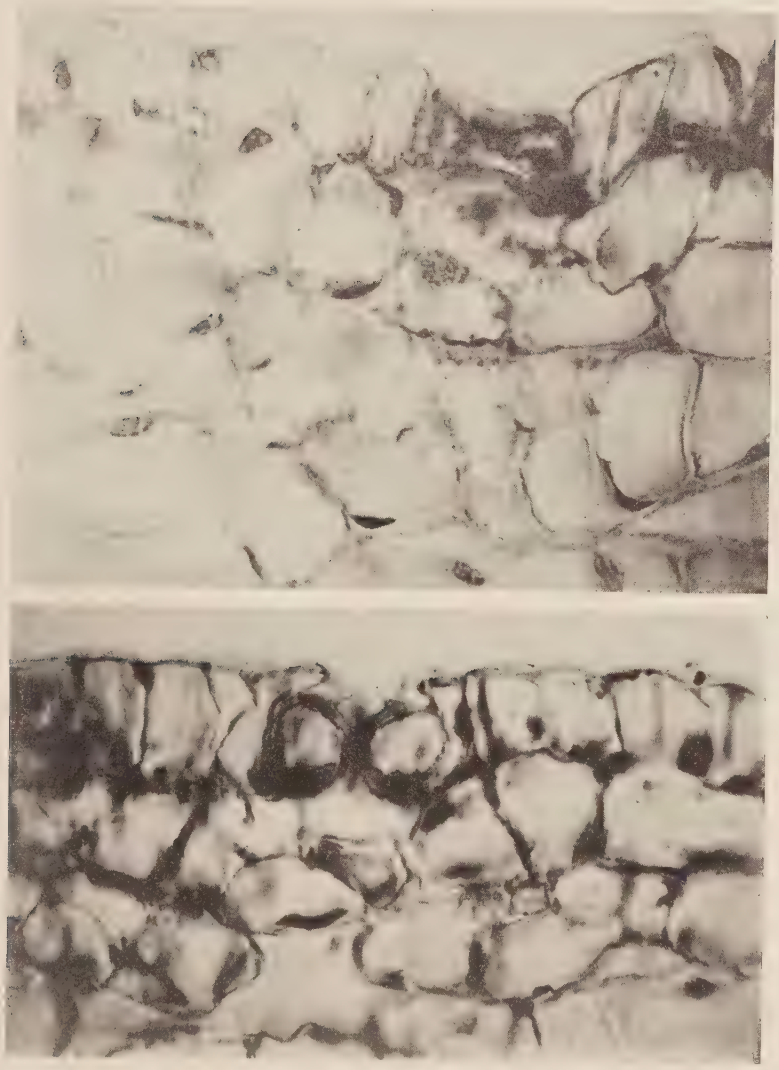


Figure 7. (Above.) Longitudinal section through a normal Bartlett receptacle showing the nature of the cuticle and stoma on outer or side walls. Magnified 950 ×. Compare with Figure 6.

Figure 8. (Below.) Similar to Figure 7 but showing a stoma in optical section. Magnified 950 ×. Compare with Figures 6 and 9.

receptacle (Figures 7 and 8). Unlike apple blossom nectarial tissue, in which the cuticle is not so easily discerned, the cuticular covering of this tissue in pear blossoms is observable with relative ease, and the reason why it has been overlooked up to the present remains more or less obscure. It responds to cuticular stains and microchemical tests as given by Eckerson *(2)* though none of these are as convincing as the direct observation of the morphological details. In thickness, the cuticle varies from around one micron over the average epidermal cell to several microns over the upper, more or less pointed, tip of the guard cells (Figure 6), though the distinction between cellulose layers and cutin at these guard-cell tips is not very clear. The most convincing evidence for the presence of cuticle on the nectarial tissue is to be found by comparing the outer covering of the epidermal cells with either the perpendicular cross walls separating the epidermal cells from each other, or with the cuticle present on the outer wall of the receptacle. When this is done it is readily seen that the outer layer, covering the epidermal cells of the nectarial region, is much thicker and stains differently from the epidermal cell walls which are perpendicular to the surface; and, as previously noted, this layer is very similar in thickness and in staining reaction to the cuticle covering the outer or side wall of the receptacle. It is ordinarily quite refractory to stains and appears as a thick, glistening layer beneath which may be seen a thinner cellulose layer. The latter is comparable in thickness and in staining reactions to the perpendicular epidermal walls. Using thionin-erythrosin-orange-G stains[2], cellulose layers stain reddish orange while cutin layers stain very faintly yellowish pink or, as more frequently happens, show no color.

The structures which serve as passageways for the nectar, previously termed nectarthodes, are structurally so similar in appearance to ordinary stomata that their true function during nectar flow might readily be overlooked in ordinary histological sections. It has already been noted that they are frequently found

[2]This staining combination has given the writer excellent differentiation of cellular structures and is particularly valuable in differentiating the bacteria from host structures of tissues affected with fire blight. The method used is a modification and simplification of the one described by Margolena (**5**). It consists essentially of the following: Paraffin sections on glass slides are put through the usual three changes of xylol, washed or pipetted with absolute alcohol, put through three changes of 95 per cent alcohol, rinsed in water, stained in phenol thionin (made up of 0.1 gm. thionin in 100 cc. of 5 per cent aqueous phenol) for 30 minutes or longer, washed in water, placed in 50 per cent alcohol for a few seconds, then in 95 per cent alcohol for a similar period, pipetted with absolute alcohol, counterstained in erythrosin-orange-G combination (consisting of one part of saturated erythrosin in clove oil and one part of saturated orange G in absolute alcohol) for approximately one minute or longer—the time depending in part on the length of time previously used in staining with thionin—pipetted with xylol, cleared in three changes of xylol, and mounted in balsam. It has proved to be the most satisfactory differential stain for studying the activities of **Er. amylovora** within host tissues, and is much more so than iron alum hematoxylin and rose bengal-light green. It stains the bacteria brilliant bluish purple, cellulose walls orange red, host cytoplasm pink, host nucleus deep blue, and nucleolus deep red. It has been used in a limited way on fungus-infected host tissues with equally successful results. Unlike rose bengal-light green stain, the colors are relatively stable and lend themselves readily to photomicrography.

embedded or located at the base of well-defined pits of the nectarial tissue (Figures 4, 5, 6, and 9), and that under a low-powered binocular miscroscope, the nectar may be observed flowing out through the openings which they make. The guard cells appear to be fully as regulatory in opening and closing the passage as those partaking in the passage of air or water vapor. In fact, it is not certain that, in addition to being secretory, they may not serve at times for the passage of air; and it is quite conceivable that following nectar flow and after the tissue has aged, they may serve as ordinary stomata.

Figure 9. Free-hand longitudinal section of Bartlett receptacle in the nectarial region showing the S-shaped figures made by the outer walls of the nectarthodal guard cells. Magnified 440 ×.

These nectarthodes are to be found at various levels below the surface, though at times they are fully at surface level and sometimes even at the tops of portions that are raised above the surrounding level. The surface of the nectarial tissue as a whole is quite irregular, and in addition to the gradual curvature upward from the region of the stylar emergence, there is an irregular series of raised and lowered portions with intervening level or flat surfaces. At times the pits are very deep with the nectarthodes located several layers of cells below the adjoining level. Often, however, the pits are shallow, with the guard cells of the nectarthode at the level of the first or second layer of cells below the epidermis (Figures 5, 6, and 9). The epidermal cells bordering the guard cells curve more or less sharply downward, the curvature involving both a shortening in diameter of the

longitudinal wall closest to the guard cell and also a partial bending downward of the whole cell toward the guard cell.

The guard cells, observed in median cross sections cut radially to their longest diameter, possess the characteristic heavy walls and sculptures of ordinary stomatal guard cells found on outer receptacle walls of pear blossoms, and are not much different in size from the latter. They possess the usual more or less pointed projection on the upper part of the wall bordering the opening, and this wall continues downward somewhat as an S-shaped figure, with the curvature varying according to the width of the opening as in air passageways.

Immediately below the guard cells of these nectarthodes, there are open areas, varying considerably in size, and corresponding to sub-stomatal chambers. When sectioned during nectar flow and mounted in Amann's fluid, these chambers as well as the passageway between the guard cells, are often seen to be filled with a more or less adhesive, and somewhat gummy fluid with a surface tension apparently differing from that of the mounting medium. Even in fixed and microtome-cut sections there are in some instances indications of an adhesive fluid occupying the chamber and passageway. At any rate, with thionin-erythrosin-orange-G stains there occasionally appears a dense dark-purplish coloring within the chamber and passageway and, at times, filling the pit above the nectarthode (Figure 6). Apparently this substance contained in nectar is not wholly soluble in water or it would have disappeared somewhere in the killing and fixing or in the staining procedure. Judging by the color reaction to the stains used, it appears to be at least in part proteinaceous in nature, and while nectar is known to be made up mostly of varying concentrations of sugars, the staining reaction suggests that it contains some complex nitrogenous materials which, with 70 per cent alcohol, formalin, and acetic acid as killing agent, presumably precipitate or coagulate out of solution or at least in part become fixed. This dense purplish coloring of the chamber below the nectarthode, within the passageway between the guard cells, and in the pit above the nectarthode is not only found in parts of the nectarial region of uninfected pear blossoms, but is more frequently to be found in blossoms infected with fire blight, as will be more fully noted later. The presence of fire-blight bacteria in the nectar presumably causes the nectar to adhere more readily to the tissues, and is not so easily washed away in killing and staining procedures.

Below the epidermal layer within the nectarial region there is a zone of tissue 12 to 15 layers of cells deep, which, morphologically, is quite distinct, and physiologically probably functions in the manufacture and movement of nectar. This tissue is composed of relatively small cells containing, at times, heavily stain-

ing cytoplasmic contents and nuclei which are exceptionally large in proportion to the size of the cells. The cell walls are very delicate and often stain so lightly that they are hardly observable in contrast to the well-defined walls of cells that are below this tissue or to the sides. The walls of the non-nectar-bearing tissue

Figure 10. Natural fire-blight infection of a pear blossom—discolored and withered—showing the independence of blossom infection from that of adjoining leaves or spur. Photographed April 27, 1934; representing the first blight detected in that year.

are, without much doubt, in the nature of cellulose, while the chemical nature of the very thin walls of the nectar-producing tissue can only be conjectured. As they show no secondary thickening during nectar flow, they probably contain little or no cellulose and they stain so delicately that they are barely observable either in free-hand sections of living tissue or in stained preparations of killed and fixed tissue. Only when the protoplasts are plasmolyzed are they differentiated with any degree of clarity (Figure 6). The protoplasts show much variation in staining reactions. Often they stain very densely, as already noted, but at other places within the same region, the density and evenness of the stain is considerably reduced, indicating possibly a difference in function. It may be surmised that when they show a heavy staining reaction, they represent cells actively engaged in secreting nectar, and when they stain lightly, they are representative of cells that for the time being are not functioning in this manner. Also, there is a possibility that within the nectarial tissue there are areas which do not take part in the manufacture and movement of nectar. In any case, this very compact tissue, possessing rather small intercellular spaces, forms an exceptionally receptive region for the growth and multiplication of *Er. amylovora,* although the larger cells and larger intercellular spaces of the tissue beneath it usually offer better optical views of the bacterial strands.

In order to study the mode of penetration of *Er. amylovora* into pear blossoms under controlled conditions and to determine the amount of invasion in given intervals of time, it is obvious that recourse must be had to artificial inoculations with pure cultures of the disease producer, and while artificial inoculations and infections are likely to introduce factors that are foreign to natural infections, nevertheless, it is clear that the chances of finding very young natural infections, so as to determine the place and manner of entrance, are limited, and when found would at best give no exact information as to the time elapsed since the bacteria were introduced. This in turn may seriously interfere with studying the mode of penetration. When it is recalled that bacteria not only enter but also pass out or exude from infected areas after varying intervals of time, and that such exudations through certain structures may be confused with passage in the opposite direction, it is clear that artificial infections offer much superior material for study so far as the important points involved in the mode of entrance are concerned. Also, it may be worth mentioning that natural infections directly through pear blossoms without recourse to the inward passage of bacteria from leaf or twig to spur and then to blossom are very common (Figure

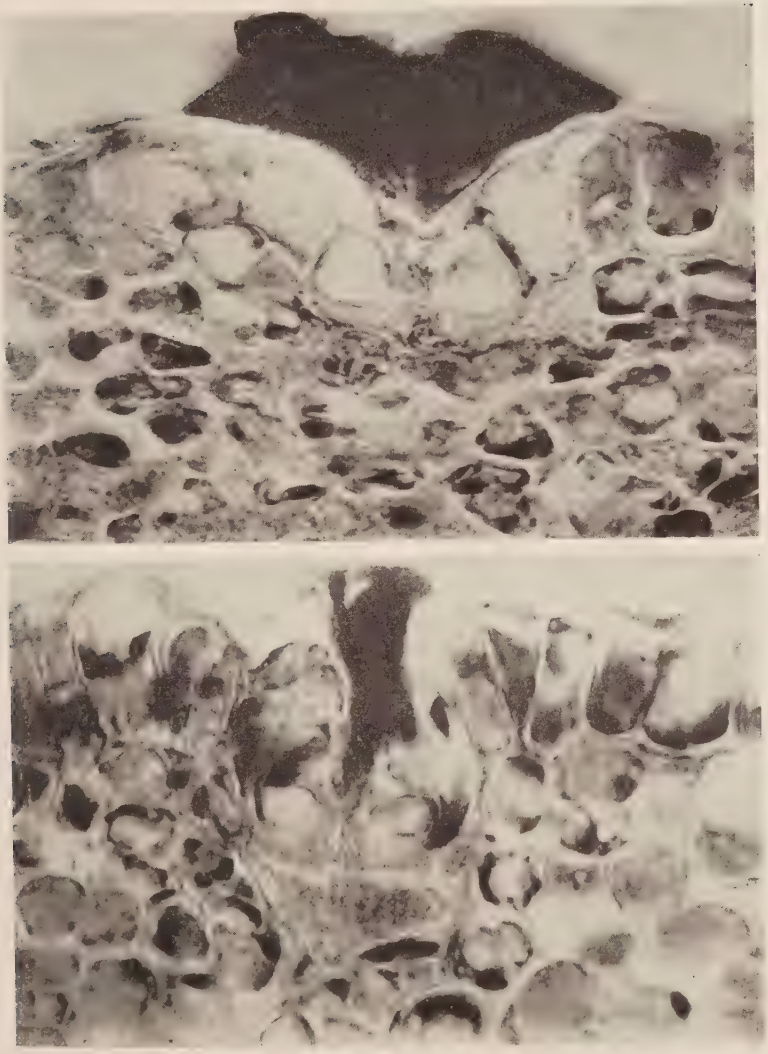


Figure 11. (Above.) Longitudinal section through the nectarial region of a Bartlett blossom, that was artifically inoculated 48 hours previous to the preparation of the blossom for study, showing the very dense, heavily-stained bacterial growth above a nectarthode, indicating the multiplication of the bacteria in a droplet of nectar. Magnified 950 ×.

Figure 12. (Below.) Similar to Figure 11, and showing the passage of fire-blight bacteria from the pit above the nectarthode, through the opening made by the guard cells, and into the sub-nectarthodal chamber. Magnified 950 ×.

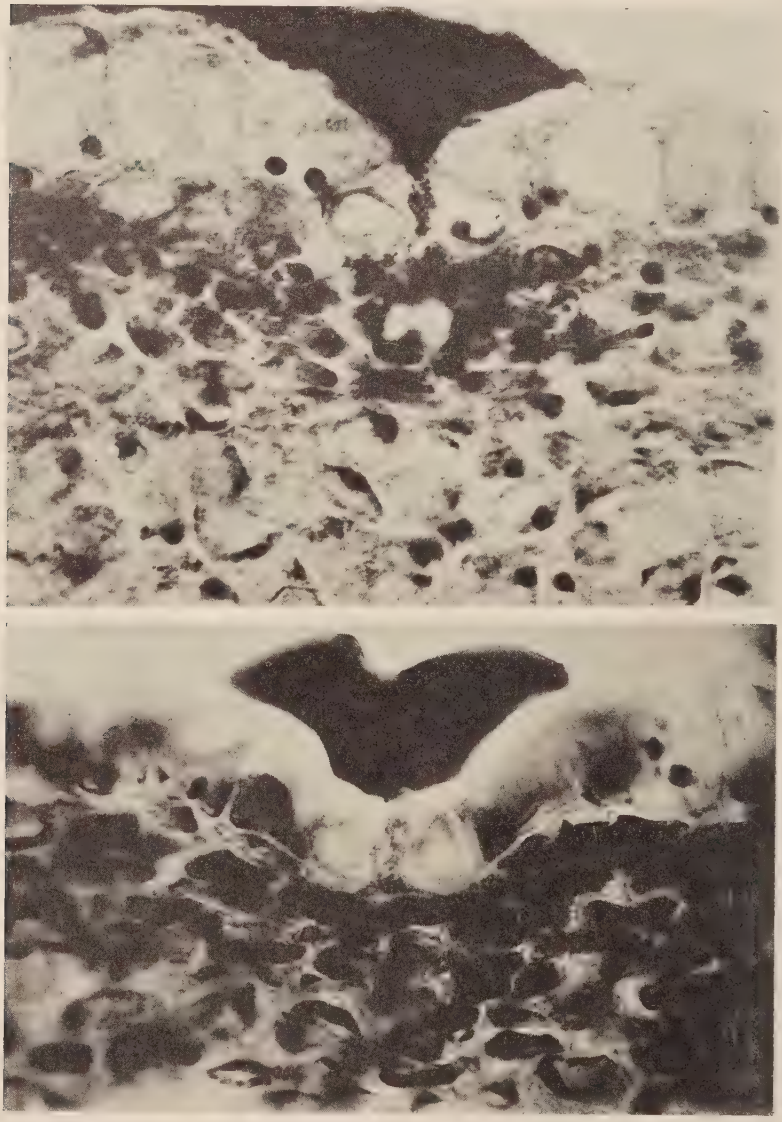


Figure 13. (Above.) Progressive invasion from dense bacterial surface growth through the nectarthode into the reduced sub-nectarthodal chamber, and into the adjoining tissues. Large, round, blackish-appearing bodies represent the nuclei of host cells. Magnified 950 ×. Material comparable to that shown in Figure 11.

Figure 14. (Below). Section comparable to Figure 11, but showing a more advanced stage of infection and parasitism as revealed by the intense discoloration of the host protoplasts, involving at least six layers of cells below the sub-nectarthodal chamber, which is much reduced in this instance. Magnified 950 ×.

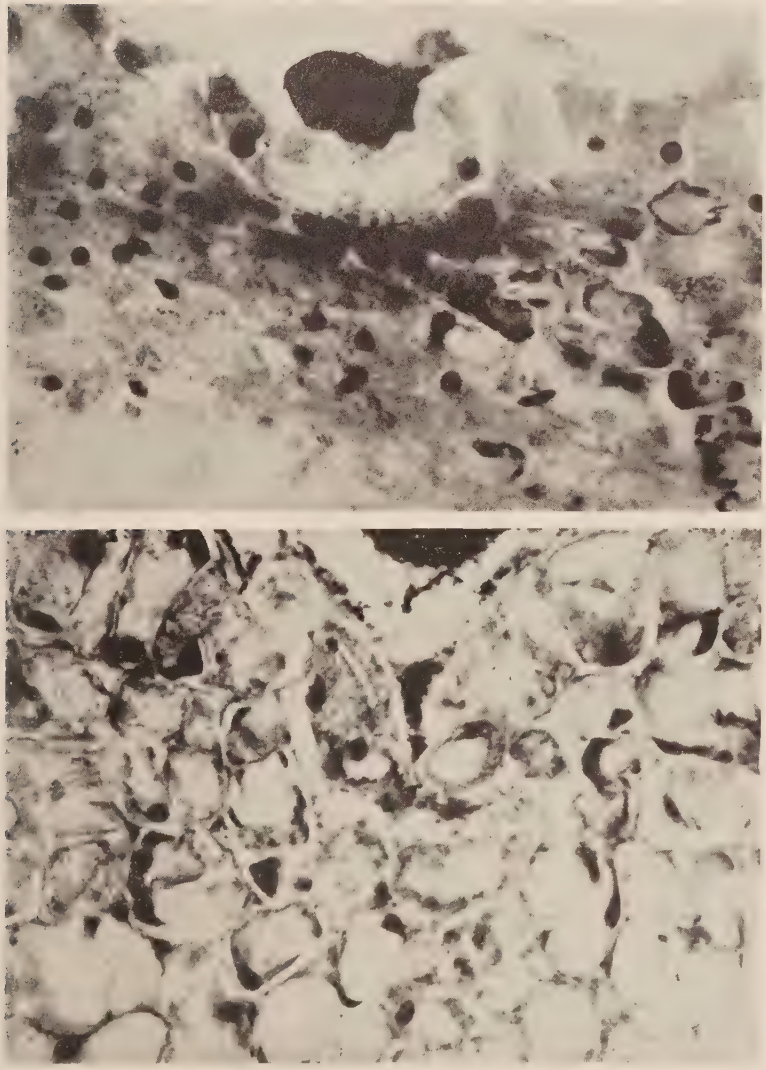


Figure 15. (Above.) Similar to Figure 11 and 13, but showing the dense discoloration of the host protoplasts in the maximum region of infection, which in this instance is not as far advanced as that shown in Figure 14. Magnified 950 ×.

Figure 16. (Below.) Similar material to that shown in Figure 11, showing dense masses of bacteria passing through a nectarthode and into the subnectarthodal chamber. The separation of the main mass of bacteria from that in the passageway is probably an artifact induced in the process of sectioning or staining. Magnified 950 ×. See Figures 18 and 19.

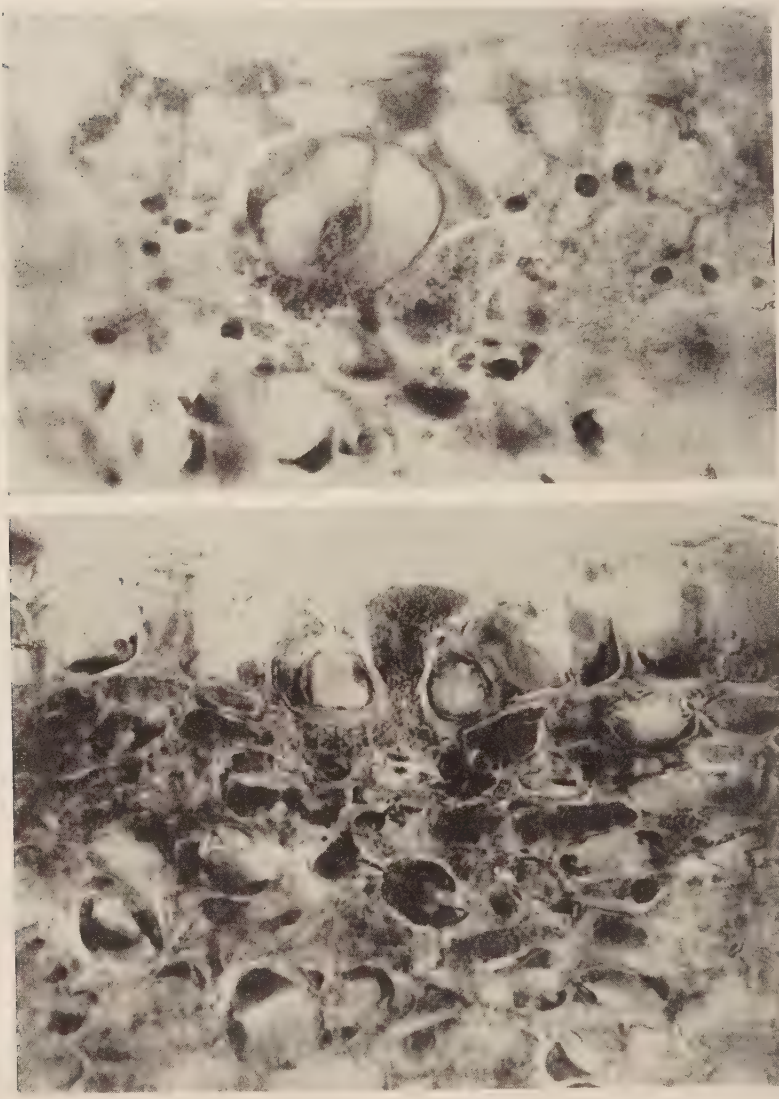


Figure 17. (Above.) An angular longitudinal section of a Bartlett nectarial disk 48 hours after inoculation showing the penetration of bacteria through a nectarthode in partial surface view, and their entrance into the sub-nectarthodal chamber and adjoining tissue. Magnified 950 ×.

Figure 18. (Below.) Similar to Figure 16, but showing the continuous mass of bacteria reaching from the surface into the interior through a nectarthode. Magnified 950 ×.

10), and that the artificial infections obtained by the writer closely resemble the natural ones.

Young vigorously-growing nutrient dextrose broth cultures, diluted with sterile water, were sprayed on Bartlett and Kieffer pear blossoms shortly after they had fully opened. The inoculations were made both on blossoms in the orchard and on others borne on severed twigs with bases steeped in water in a greenhouse. Controls consisting of blossoms sprayed with diluted sterile nutrient broth were maintained both in the field and greenhouse, and glassene bags were tied over all inoculated and control blossoms for 24 to 48 hours following inoculation in the orchard. No difference in mode of penetration was found in the blossoms inoculated in the orchard compared with those inoculated in the greenhouse.

Within 24 to 48 hours following inoculation, the bacteria are found not only on the nectarial surface but penetrating to various depths into the receptacle tissue. There is excellent evidence indicating a rapid multiplication of bacteria in the drops of nectar located above nectarthodes (Figures 11, 12, and 13). In the pits above these structures the bacteria are often to be found in such great abundance, quite frequently filling them so closely and heavily, that in differential staining with thionin erythrosin orange G, these pits stain densely purple and are so full of bacteria that individual organisms are rather difficult to distinguish at times. So closely do the bacteria fill the nectarial fluid that it suggests the possibility of tracing the outline of the fluid by the dense masses of bacteria (Figures 11, 14, and 15).

From the mass of organisms gathered above the nectarthodes, extensions can frequently be traced through the openings made by the guard cells, reaching into the interior chambers below the nectarthodes (Figures 16, 17, 18, and 19). Indeed, while the nectarthode is secreting nectar, the nectar above the passageway is probably continuous with that in the chamber below and would, therefore, offer a very favorable condition for the bacteria to pass from the outside nectar through the passageway made by the guard cells and into the nectar-filled chamber below. It would be difficult to conceive of a more favorable condition for the entrance of bacteria through a natural opening of susceptible tissue, and probably explains why the nectarial tissue of pear blossoms is more frequently found serving as points of entrance, at least in artificial infections, than any other part of the blossom.

This mode of invasion through pear nectarthodes by *Er. amylovora* is somewhat comparable to that described by Meier *(6)* for the entrance of *Phytomonas campestris* into cabbage hydathodes, in which she found a continuous pathway of liquid reaching from tracheid tips to the hydathodes. Meier, reporting that the multiplication of the bacterial mass appears to be in the direc-

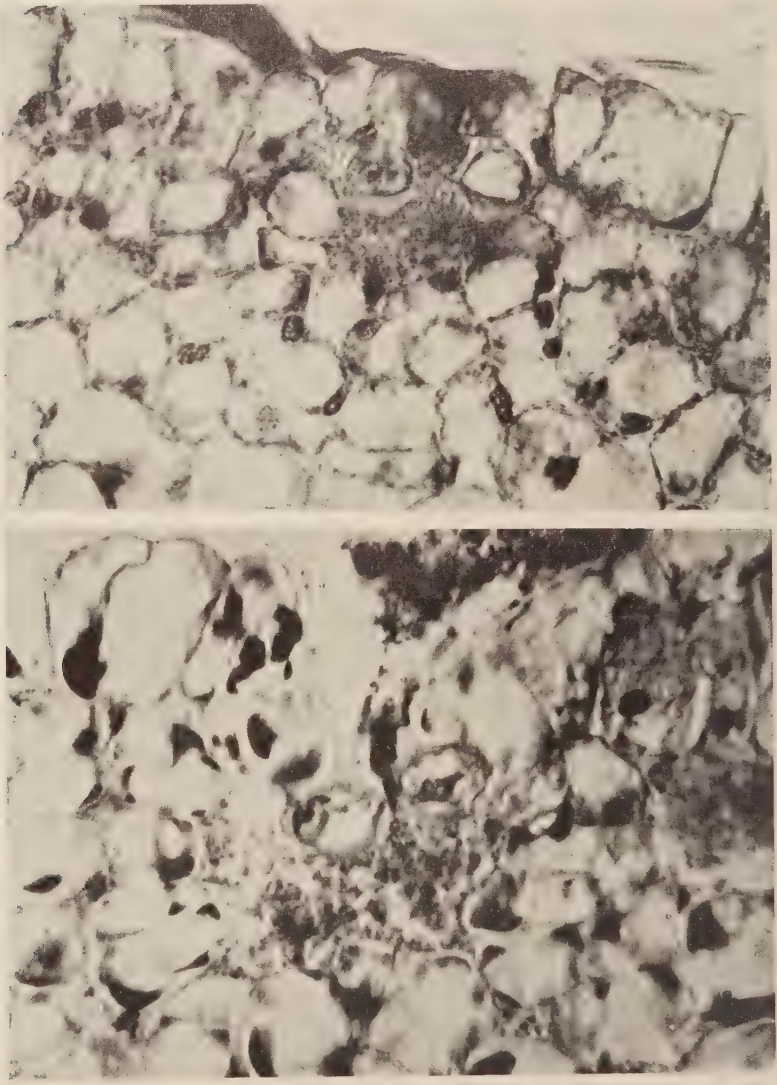


Figure 19. (Above.) Similar to Figure 18, but invasion and parasitism has not as yet advanced beyond the sub-nectarthodal chamber to any great extent. Note that the host protoplasts are much more lightly stained and that one or more of the nuclei in focus are still sharply delimited. Magnified 950 ×.

Figure 20. (Below.) Longitudinal section through a Bartlett receptacle in the nectarial region showing the advance of bacteria from the sub-nectarthodal chamber into the tissue surrounding the chamber. Note the presence of bacteria in several intercellular spaces, located one layer of cells below and to the left of the chamber. Magnified 1150 ×.

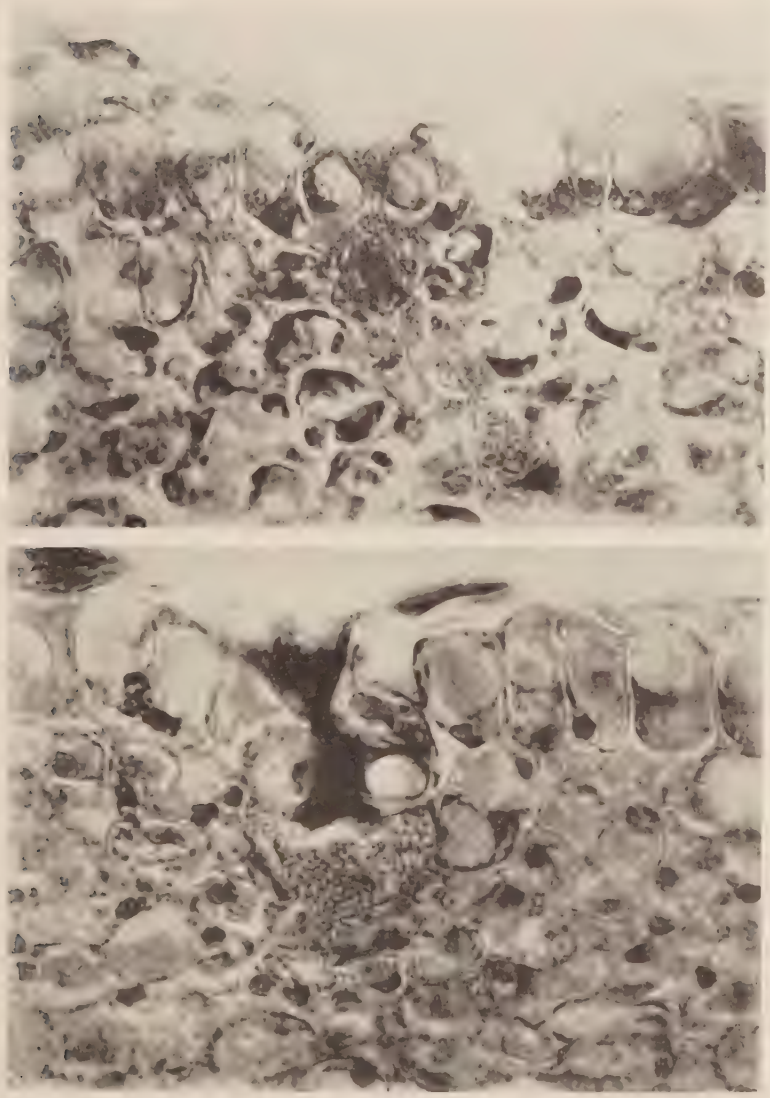


Figure 21. (Above.) Longitudinal section through Bartlett nectarial tissue 48 hours after inoculation showing the advanced penetration of bacterial strands from the surface to several layers of cells below the sub-nectarthodal chamber, the passage being mainly between the cells. Magnified 950 ×.

Figure 22. (Below.) Similar to Figure 21, but the invasion from sub-nectarthodal chamber is not so far advanced. Magnified 950 ×.

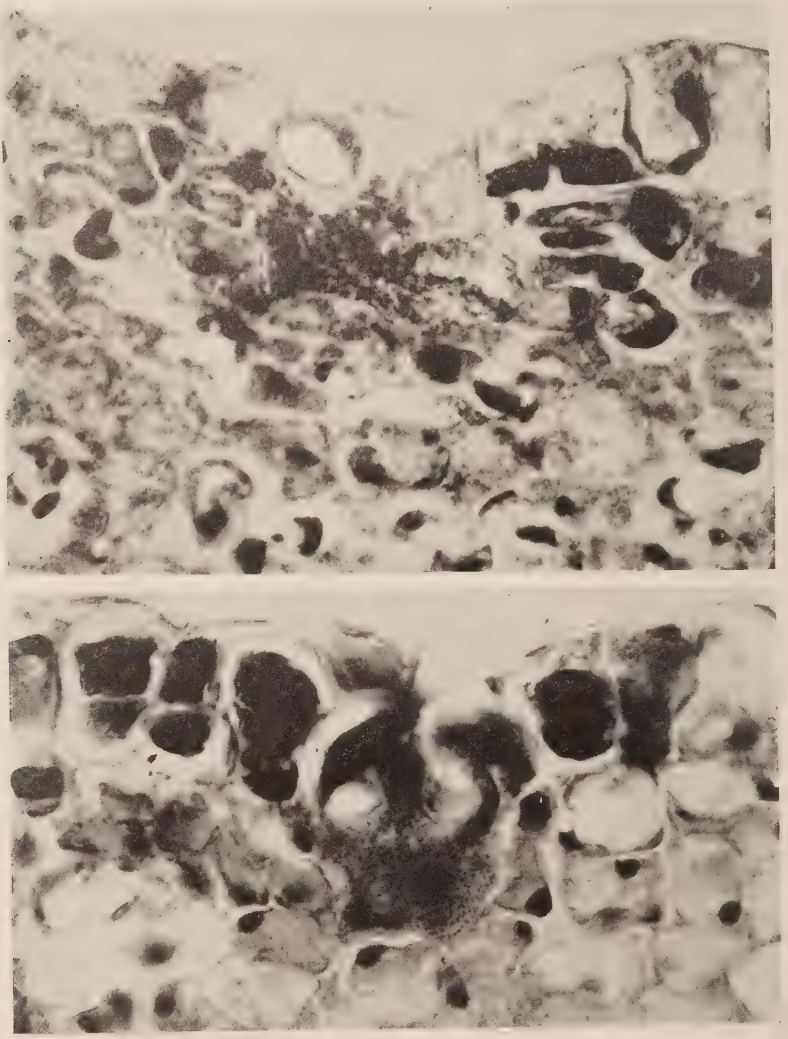


Figure 23. (Above.) Intense discoloration and coagulation of some of the host protoplasts in the region bordering the sub-nectarthodal chamber in 48-hour-old infection of Bartlett receptacle. Magnified 950 ×.

Figure 24. (Below.) Discoloration and coagulation of the protoplasts of epidermal cells in 48-hour-old nectarthodal infection. Magnified 950 ×. Note that the protoplasts around the sub-nectarthodal chamber do not in this instance show as much destruction as those of the epidermis, suggesting that in this particular invasion, the bacterial mass in the chamber extended first in the direction of the epidermal layer.

tion of the food supply, stated, "As the food is exhausted near the hydathode, multiplication of the bacteria takes place in the direction of the tracheid tips." This investigator further reported, "In their passage from the hydathodes to the tracheid tips the bacteria travel only in the epithem region where a fluid pathway is present."

The entrance of fire-blight bacteria into the nectarthodes and into the chambers below may be considered as a phenomenon involving simply the saprophytic growth of these bacteria in the presence of a medium highly conducive to their multiplication. However, as the bacteria multiply in the sub-nectarthodal chambers, they doubtless fill these chambers with their waste products and enzymes, and it is quite probable that the progressive invasion from these points is concomitant with the first parasitic expressions of these bacteria. In other words, it seems likely that any microorganism capable of growing in pear nectar which may have lodged on the exposed fluid may reach as far as the sub-nectarthodal chamber, but from that point, the advance would at least in some measure depend upon the kinds of metabolic products and enzymes which the invader is capable of producing. If these materials are permeable and toxic to pear protoplasts, as in the case of *Er. amylovora*, the invader gives rise to parasitic expressions and progresses further on into the tissues. While there can be little doubt that Waite *(17)* missed the actual mode of entrance of the fire-blight pathogen into the nectarial tissues of pear and apple blossoms, it is only fair to call attention to the remarkable insight he displayed in definitely noting in 1891 the saprophytic multiplication of the bacteria in the nectar.

At the end of 48 hours fire-blight bacteria fill not only the chamber below the nectarthode, but may be traced at times passing between cells and into intercellular spaces for a distance varying from one to eight layers of cells below or to the sides of the sub-nectarthode chamber (Figures 20, 21, and 22). The mode of tissue invasion involves, at first, seemingly a mere extension of the bacterial mass occupying the nectar chamber, accompanied by marked discoloration, plasmolysis, coagulation, and disintegration of the protoplasts of cells adjoining and in the immediate vicinity of the chamber (Figures 12, 13, 15, and 23). Often the epidermal cells bordering the nectarthode show a greater intensity of discoloration (Figure 24) than the cells below or at the sides of the chamber, though this is not always the case. It is this intense discoloration of the protoplasts of the epidermal cells which makes an infected receptacle cup appear black in gross observations. However, nearly all protoplasts of cells, several layers deep, surrounding the chamber soon show marked discoloration and plasmolysis (Figures 14 and 25) suggesting a very intense

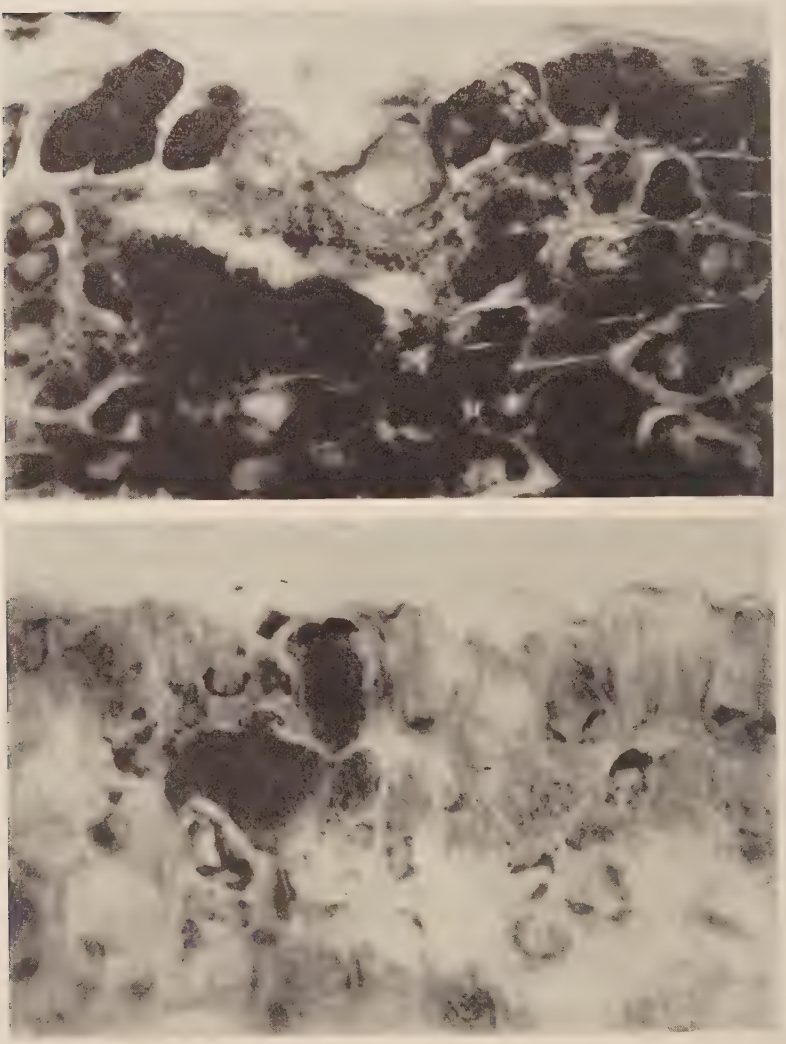


Figure 25. (Above.) Extensive discoloration and coagulation of host protoplasts in 48-hour-old nectarthodal infection of Bartlett pear. Magnified 1000 ×.

Figure 26. (Below.) Bacterial invasion of protoplasts in 48-hour-old nectarthodal infection of Bartlett pear. Magnified 1000 ×. Note particularly the internal invasion of an epidermal cell bordering a nectarthode, and the extension of the bacterial mass from the sub-nectarthodal chamber into the epidermal cell, with a marked suggestion of passage into the cell, not by mechanical pressure but by a localized dissolution of cell wall. If mechanical pressure were involved, one would expect a distortion and pushing in, or convexity of the portion of the wall impinging on the bacterial mass.

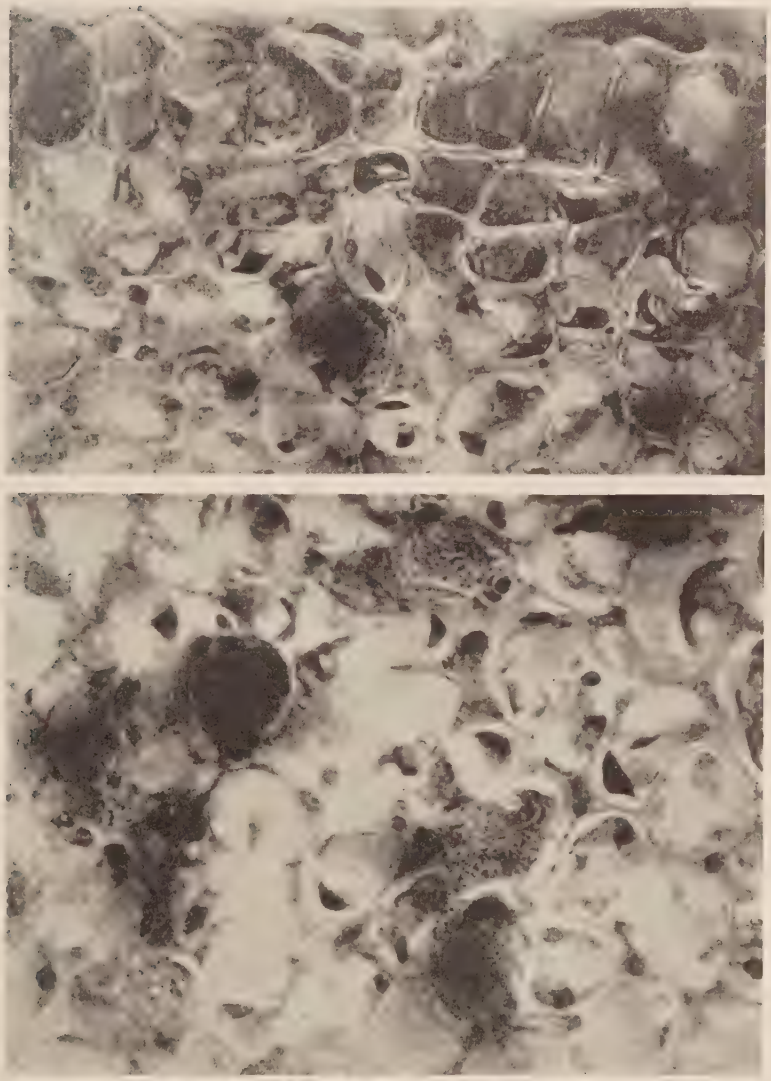


Figure 27. (Above.) Bacterial migration in Bartlett nectarial tissue 48 hours after inoculation, showing inter- and intracellular invasion. Magnified 950 ×. Intercellular spaces in this young, partly-embryonic tissue are mostly small.

Figure 28. (Below.) Longitudinal section of Bartlett nectarial tissue 48 hours after inoculation, showing penetration of protoplasts, as well as intercellular migration. Magnified 1425 ×.

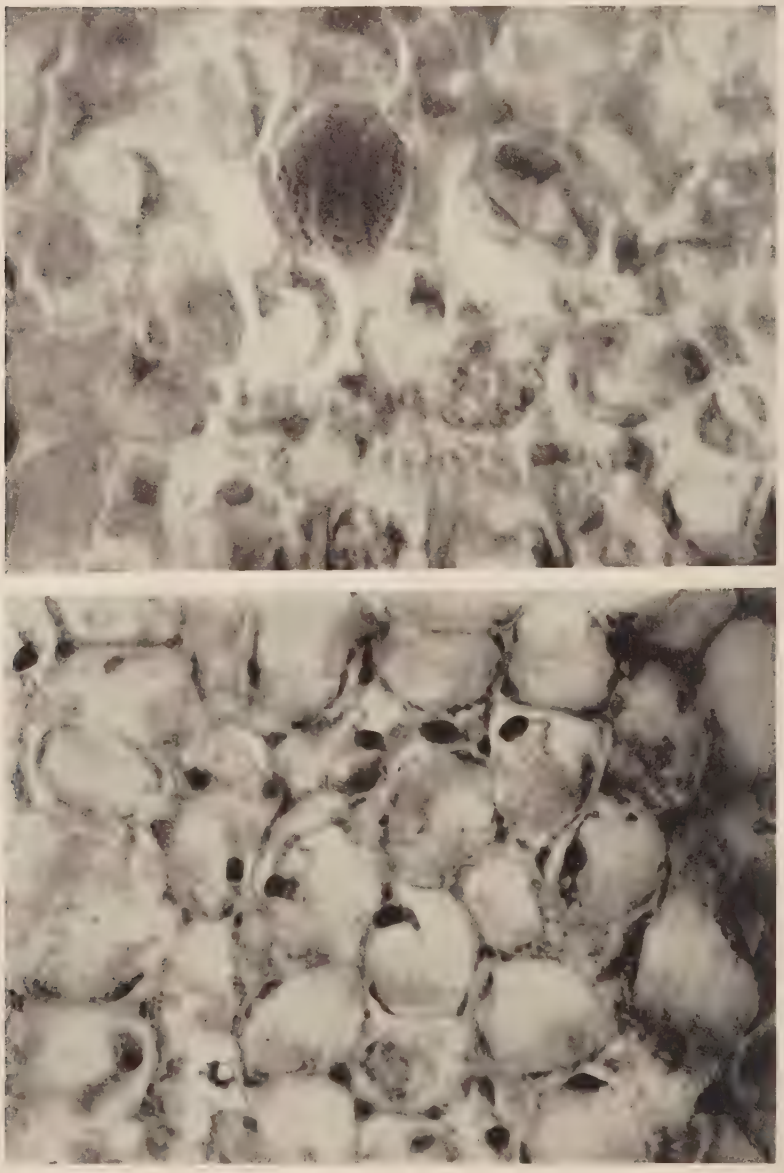


Figure 29. (Above.) Combined inter- and intracellular migration of bacteria in Bartlett nectarial tissue 48 hours after inoculation. Note the bacterial occupation of an intercellular space and of an adjoining cell. Magnified 1450 ×.

Figure 30. (Below.) Intercellular migration of bacteria in Bartlett nectarial tissue 48 hours after inoculation. Magnified 1450 ×.

oxidation phenomenon such as the writer has previously described for infected pear petals *(11)*.

Not only do the bacteria progress in the tissues through the intercellular spaces, as Bachmann *(1)* first adequately described for this organism, but there is excellent evidence indicating a

Figure 31. Longitudinal section of Bartlett pear receptacle below the nectarial region, 48 hours after inoculation, showing the intercellular migration of bacteria. Magnified 1450 ×.

localized dissolution or disruption of middle lamellae and of the delicate walls, characteristic of the cells of the nectarial tissue, and an invasion of the protoplasts (Figures 26, 27, and 28). There can be no question of the invasion at times of the protoplast itself within 48 hours after inoculation, since the coagulated, shrunken protoplast is to be noted occasionally at the same optical level as the bacterial mass, and the regularity of the bacterial mass within the cell walls leaves little doubt that they made their

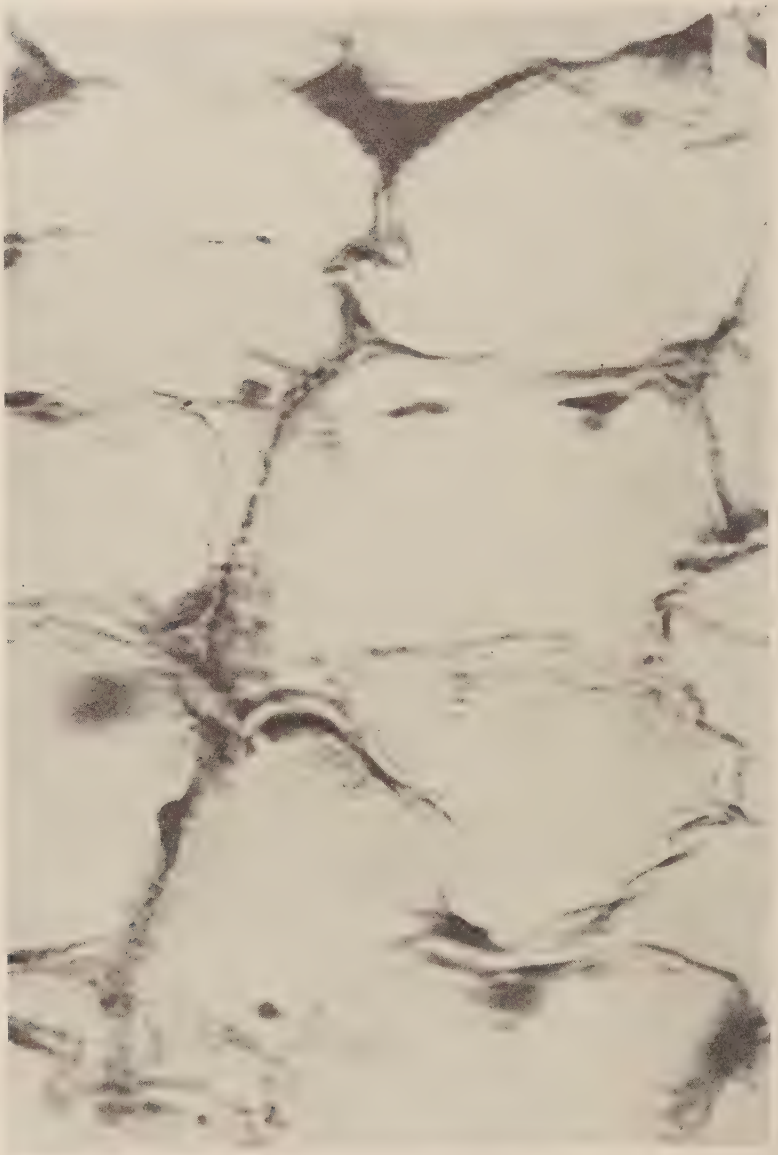


Figure 32. The nature of bacterial strands in the tissue below the nectarial region connecting with larger masses of bacteria in intercellular spaces of Bartlett receptacle, 48 hours after inoculation. Magnified 2472 ×.

way into such cells, and were not merely carried there as artifacts induced by sectioning and staining (Figures 26, 28, and 29).

Various theories have at different times been proposed to explain the manner in which the bacteria separate or disrupt cell walls, including mass action, osmotic pressures, and chemical or enzymatic hydrolysis and dissolution. The writer has found no evidence in his studies which would unqualifiedly favor any one of these theories, though there is a suggestion in favor of enzymatic dissolution because of a slight difference at times in staining reaction of the walls of cells impinging on bacterial masses. This difference, however, is not clear cut, and it is difficult to understand why dissolution is so localized if it is due to the action of an enzyme, such as pectinase, cytase, or cellulase. According to Pitman and Cruess *(10)*, *Er. amylovora*, compared to various other microorganisms, has little effect on pectin when it is incorporated into a culture medium. However, they found that in the presence of this organism the pectin loses some of its jellifying power, the viscometer reading is reduced, and 8.26 per cent is hydrolyzed. This is in direct contrast with the finding of Pierstorff *(9)* who placed thin slices of unripe pear and apple fruits in bouillon cultures of the fire-blight organism and in boiled as well as in fresh juices which were extracted from blighted pear fruits, and observed no dissolution of the middle lamella "prior to disintegration of the entire tissue." He likewise prepared a medium consisting of Uschinsky's solution, lacking glycerine, and a commercial pectin-containing substance, Certo—the formula or the kind of Uschinsky's solution used is not given—and inoculated the medium with *Er. amylovora*. No reducing sugars or coagulation were noted, and he concluded that "under the conditions of the tests neither pectase nor pectinase was produced." These are apparently safe conclusions since certain kinds of Uschinsky's solution permit little or no growth of this organism, as noted by E. F. Smith (*15*, p. 371) and as confirmed by the writer. The only kind of Uschinsky's solution which permits fair growth of this organism is one that contains organic nitrogen, such as sodium aspariginate or peptone, and even with these, this organism does not grow as well in such synthetic media as various other bacterial plant pathogens. Likewise, the influence of the type of medium utilized for culture purposes on the production of enzymes is ignored by Pierstorff. His negative tests for the production of cellulase by *Er. amylovora* are open to the same objections.

Cellular invasion is found in greater abundance in the nectarial tissue than in the regions below or at the sides, although even in the nectarial region, the invasion is more frequently intercellular than intracellular (Figure 30). As the invasion progresses beyond the nectar-secreting tissue, the passage of bac-

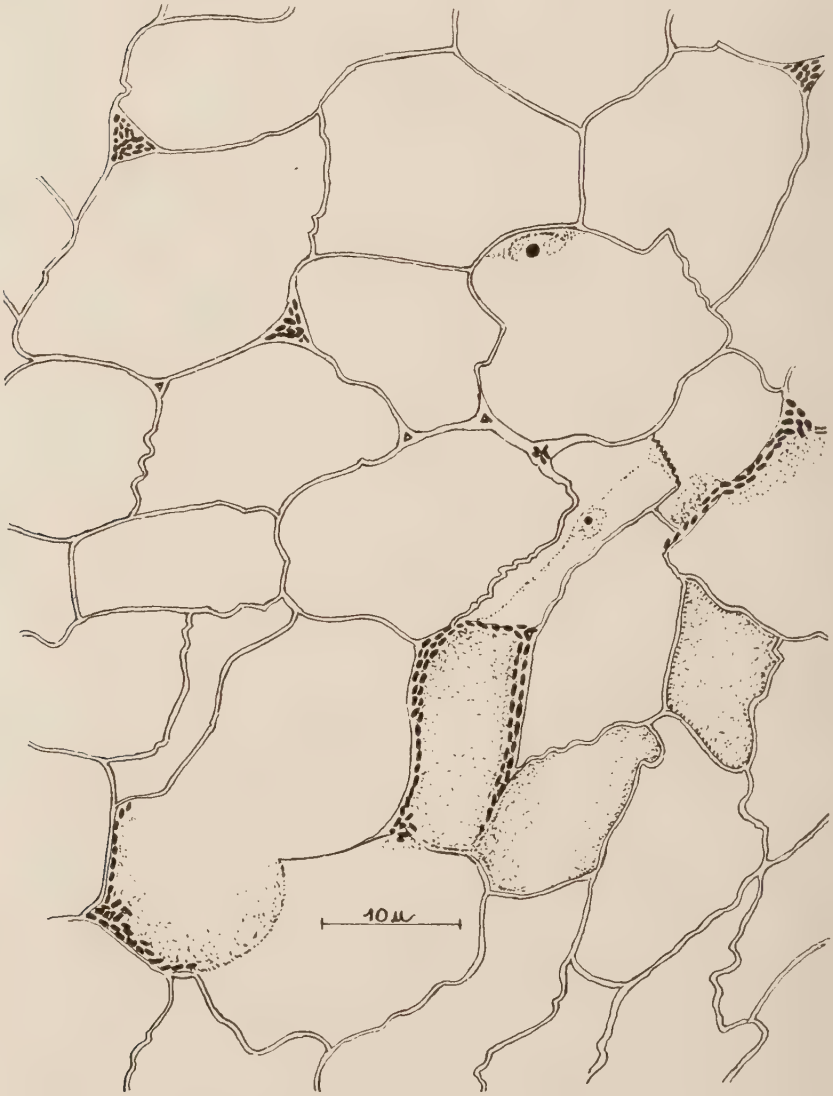


Figure 33. Bartlett receptacle, 48 hours after inoculation, showing bacterial penetration involving tissue located 22 to 27 layers of cells beneath the nectarial surface, and suggesting a manufactured passage between cells as shown in the one surrounded by bacteria on three sides.

teria is largely confined to intercellular spaces (Figures 31 and 32), and to passageways apparently made by the bacteria between the cells. The evidence for the manufactured passageway between cells rests upon the fact that bacterial strands may more or less frequently be observed impinging on two or more sides of any one cell (Figures 33, 34, and 35), and so surrounding it as to isolate it more or less completely from adjoining cells. Likewise, the bacterial mass starting from a well-defined triangular-shaped air space may occasionally be seen in single file making their way between adjoining cells where there are no indications of intercellular spaces (Figures 36, 37, and 38). There seems to be little doubt that the bacteria can dissolve middle lamellae at times, though their abilities to do so are much more limited than in such distinctive soft-rot producers as *Erwinia carotovora* or *Phytomonas dissolvens*. However, at times they make their way between cells in such thick strands and in such enormous numbers (Figures 39 and 40) that it can readily be understood why they show such aggressiveness in producing disease in soft tissues.

The mechanics of bacterial advance or migration from place to place within the tissue is in open question, though the writer's studies have seemingly clarified certain essential details that must be considered in evaluating the different theories concerning bacterial migration. The studies have shown that bacterial invasion is not simply a matter of change in place of one organism or group of organisms. The bacterial strands in serial sections are readily traceable in continuous threads in infected pear blossoms, reaching from the mass of surface bacteria growing in the nectar, through the nectarthode, to various depths within the tissue. In other words, assuming that the number of bacteria developed within 24 hours in a drop of nectar hanging over a nectarthode is 500,000, the migration or advance into the tissue apparently is not due to the movement of 100,000 or more from the surface into the interior, leaving few or none behind, but the evidence suggests that the advance is due to the addition or accretion of additional numbers to the bacterial mass, with a consequent spreading or enlargement of the area occupied by the bacteria. In the presence of an open nectarthode containing or secreting nectar, the spreading of the bacterial mass into the sub-nectarthodal chamber offers no essential physical or chemical difficulty and, on the other hand, the nectar pathway is more likely to favor growth and expansion of the bacterial mass in that direction.

After the bacteria have multiplied to any considerable degree in the nectar and have advanced into the tissue, there is no evidence to indicate that the individual bacteria are motile. Indeed, under natural conditions, it may be questioned whether this organ-

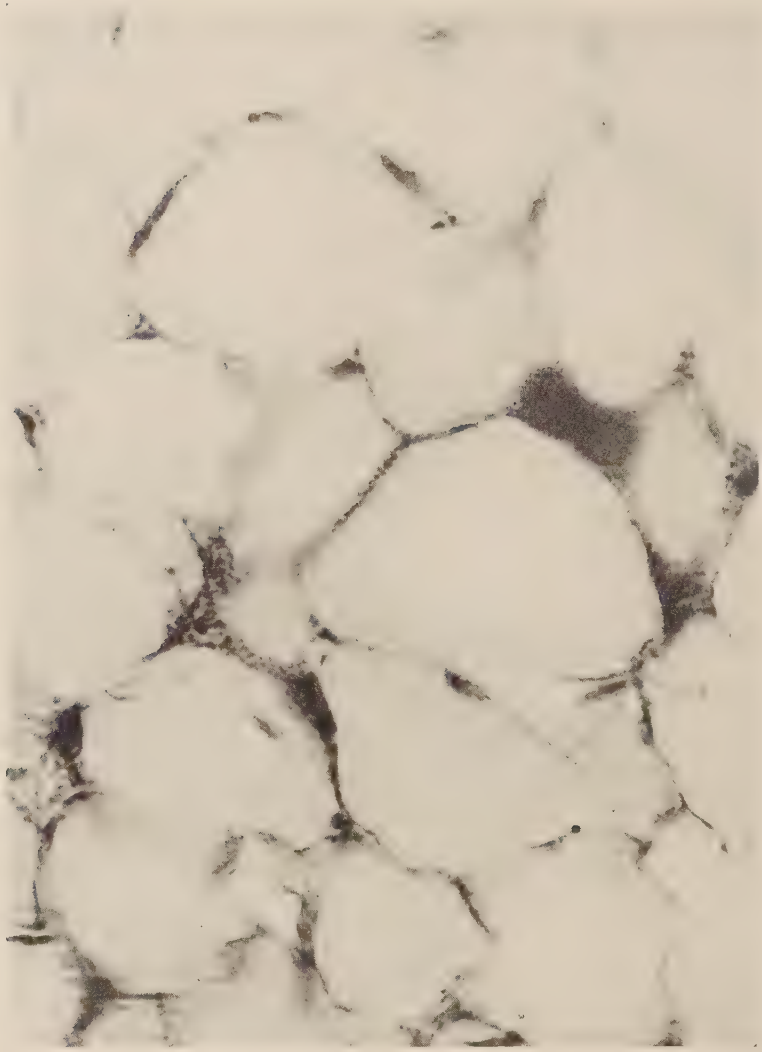


Figure 34. Bartlett receptacle, 48 hours after inoculation, showing the complete surrounding of a cell in one plane by bacterial strands deep in the receptacle, and suggesting, in part, a manufactured passageway between adjoining cells. Magnified 1350 ×.

Figure 35. Same as Figure 34 but at a different optical level, indicating the continuity of bacterial strands, and the more or less complete investment of individual host cells or groups of cells by the bacteria. Magnified 1350 ×.

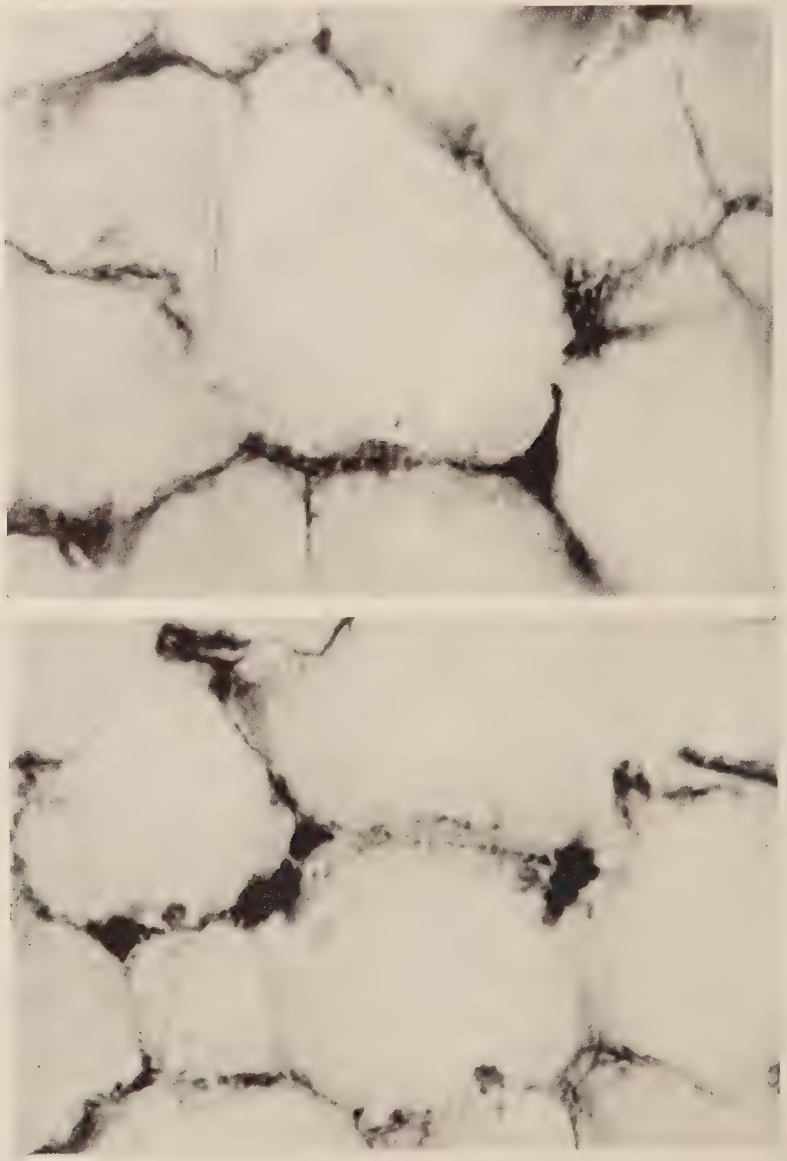


Figure 36. (Above.) Penetration of bacteria deep in Bartlett receptacle tissue, 48 hours after inoculation, showing the bacteria migrating partly in single file between cells where there is no evidence of an intercellular space. Magnified 1950 ×.

Figure 37. (Below.) Similar to Figure 36, but showing the intercellular migration of bacteria with their long axes variously disposed and not suggestive of a creeping zoogloeal movement. Magnified 1950 ×.

ism is ever motile while it is actively advancing into live nectarial tissue. When the bacterial mass, 48 hours old, contained in a nectar pit is touched with a needle, the bacteria are drawn out into slimy threads, clearly indicating the secretion of a viscid gummy substance which makes the bacteria cling together rather tenaceously, much in the same manner as that commonly shown by fresh

Figure 38. Bartlett receptacle tissue, 48 hours after inoculation, suggesting a partial dissolution of middle lamellae by the passage of bacteria between two adjoining cells. Magnified 950 ×.

bacterial exudate of *Er. amylovora*. In the presence of such adhesive slime, glueing the bacteria together, it is readily understandable why no true movement of the individual bacteria is possible. The ideas concerning motility of this and other bacterial plant pathogens are in most instances drawn from studies of the bacteria in pure cultures—often on highly artificial media which have little or no counterpart in physical or chemical composition of the living hosts—and merely express the behavior of the bacteria on dead matter contained in liquid media or on solid media suffused with water. Such media are especially likely to give false impressions relative to motility and also to the production of bacterial slime, particularly with fire-blight bacteria, since this

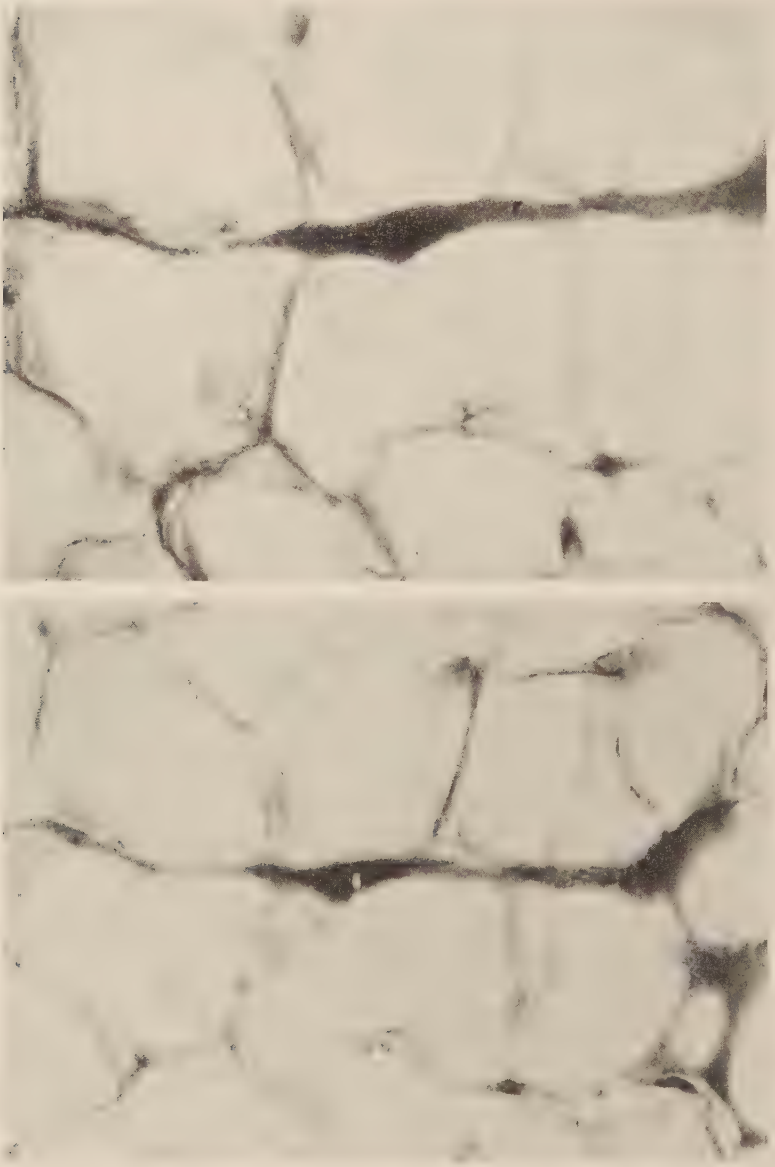


Figure 39. (Above.) Very thick intercellular rope-like strands of bacteria, commonly found deep in the receptacle tissues, representing infections that are 48 hours old. Magnified 1250 ×.

Figure 40. (Below.) Same as Figure 39 but at a different optical level, showing the branching and ramification of bacterial strands within receptacle tissue. Magnified 1000 ×.

gummy or slimy matter is more or less soluble in water, or at least deliquescent in this species, so that the slimy secretion of bacterial colonies in liquid cultures is hardly to be observed around the bacterial bodies and only to a slight degree in so-called solid media. As Shaw *(14)* has shown, no growth of *Er. amylovora* on solid media is to be had unless there is at least a 97 per cent relative humidity, which in turn means a medium containing much water. The writer has no evidence to indicate that fire-blight bacteria

Figure 41. Gross structure of an apple blossom (Jonathan) showing the nectarial region covered and concealed by the ring of stamens in the upper two views, and shown diagramatically in the lower view in a blossom cut longitudinally. The curved surface between the base of the combined styles and the points of attachment of filaments represent the nectarial tissue. Somewhat after Focke, in Engler and Prantl, Natuerliche Pflanzenfamilien 3 (3): 23. 1888.

gain entrance and progress within nectarial tissues as the result of the motility of individual bacteria.

Nixon's *(8)* interesting hypothesis of migration in host tissues by this organism concerns a movement by "zoogloea," which he considers to consist of a jelly-like mass in which the bacteria

Figure 42. Longitudinal section of a normal apple blossom showing the rather steep and curved nectarial surface. Magnified 80 ×.

are imbedded. This jelly-like mass, according to Nixon, sends out "pseudopod-like" projections which creep through the intercellular spaces. His inoculations consisted wholly of wounding water sprouts of apple with fine needles, and introducing the bacteria into the wounds. He made no attempt to study the migration through natural openings. Within 24 to 48 hours after inoculation he finds the migration to involve radially only a few cells, but tangentially the complete circumference of the stem cortex,

and longitudinally it may involve the entire water sprout, extending several feet. Nixon insists that the actual migration of the organism has been mostly overlooked by previous investigators because the material taken for study was in advanced stages of the disease. However, as he does not indicate how much, if any, tissue penetration has occurred within 24 hours after inoculation in his own studies, it is not clear how much has been overlooked. Nixon believes that there are two different kinds of migration: the one by zoogloea occurring only within a short period after inoculation; and the later one, presumably after 48 hours, by "pressure or mass action." Is it possible that the slimy envelope found in this organism when it is growing in nectar or when it is exuding from host tissue is being considered as the "jelly-like" substance by Nixon? If so, it is certainly not analagous to the complex and often beautifully symmetrical sorocarps of the Myxobacteriaceae consisting of great masses of jelly in which bacteria are imbedded, which he compares to the "zoogloea" of *Er. amylovora*. Indeed, several investigators have questioned the use of the term, "zoogloea," for this and other bacterial plant pathogens, and Nixon's citation of the observance of zoogloea by Arthur in the fire-blight organism can be attributed to unavoidable contaminations which were so difficult to overcome in the technics available to early bacteriological workers. In any case, in a personal communication addressed to the writer on June 1, 1926 (almost a year previous to Nixon's publication), Dr. Arthur wrote as follows: "You must bear in mind that I did not possess modern technic (about 1885) and so allowed the similar non-parasitic bacteria from the surface of pear fruit and twigs to enter my cultures. This did not vitiate my study of the parasitic organism, except to ascribe to it and figure the zoogloea stage, which only belongs to the non-parasitic form." The non-parasitic form to which he refers is probably the common saprophytic yellow organism frequently found not only on the surface of plant tissues but often following *Er. amylovora* within tissues killed by the pathogen.

Histological Studies of Penetration and Progressive Invasion of Apple Blossoms by Er. amylovora

Unlike pear blossoms with their exposed nectarial tissues, apple blossoms offer quite a different problem with reference to the penetration of fire-blight bacteria. There is little doubt in the writer's mind that nectarial penetration of apple blossoms has been somewhat exaggerated, largely owing to the long prevailing belief that nectarial tissues of both pears and apples lack cuticle.

A close study of the gross morphology of apple blossoms reveals that the nectarial region is so thoroughly ensconced by the

stamens (Figure 41) and by the heavy tomentum of basal stylar hairs, that the nectar-exuding tissue is more or less completely hidden from view and well shielded from meteoric water unless it be in the form of driving rains. To reach the nectar, a honey-bee extends its proboscis and often arranges its body in such a way as to give the appearance of standing on its head. This is the usual aspect of the bee when its body straddles the stamens and stigmas,

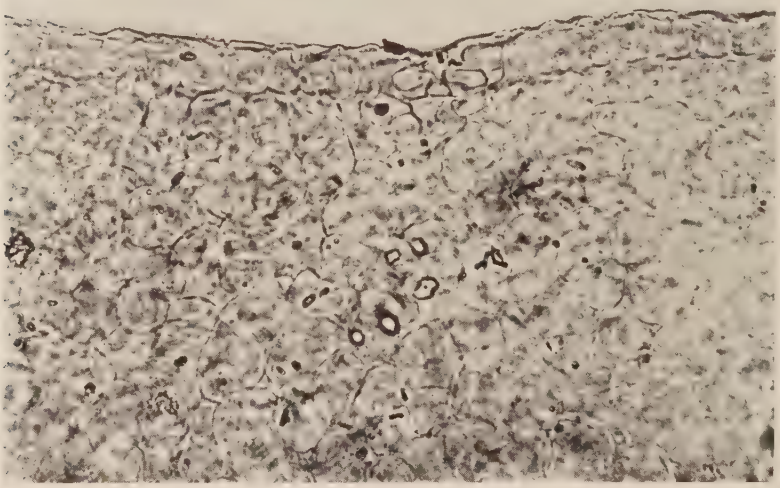


Figure 43. Free-hand longitudinal section of a normal Jonathan apple blossom in the nectarial region, showing cuticle and a nectarthode. Note cuticular sculpturing, and compare cuticle thickness with that of cell walls below the surface. Magnified 500 ×.

though it also obtains nectar by arranging its body to one side of the stamens, forcing them apart, and projecting its proboscis downward and at an angle between two filaments. These observations of the more or less completely covered nectarial regions of apple blossoms during nectar flow, compared with the exposed nectarial tissues of pear blossoms, are directly comparable with Waite's *(19)* observations. He said: "The apple blossom is formed on the same general plan as the pear flower, the only striking difference being that the bases of its stamens are enlarged and hairy, and instead of curving outward they all draw together toward the center and fit closely around the styles. By this arrangement the nectar disk is completely covered and hence only the larger insects, such as bees and bumblebees, which can force the stamens apart and reach the honey with their mouth parts can make use of the blossoms."

As in pear blossoms, the nectarial region is located between the stamens and the point of stylar emergence from the receptacle. But, unlike pear blossoms, this region, instead of being shallow and saucer-shaped, is deep and more or less funnel or cup-shaped (Figures 41 and 42). The nectarial area is arranged on a decided-

Figure 44. (Above.) Killed and stained longitudinal section of Jonathan nectarial tissue showing a nectarthode and cuticle. Magnified 600 ×.

Figure 45. (Below.) Longitudinal section of a Jonathan receptacle showing the nature of the cuticle covering of the outer or side walls. Compare with Figures 43 and 44. Magnified 750 ×.

ly sloping surface, which, at times, causes the nectar to gather in considerable quantity at the base of the slope where the stylar tube emerges from the receptacle.

Owing to the very heavy coating of hairs on outer receptacle walls and on styles of apple blossoms, good paraffin sections are rather difficult to obtain, unless these hairs are first removed. When this is done and longitudinal sections are made of the nectarial tissue, one finds essentially the same structures as in pear

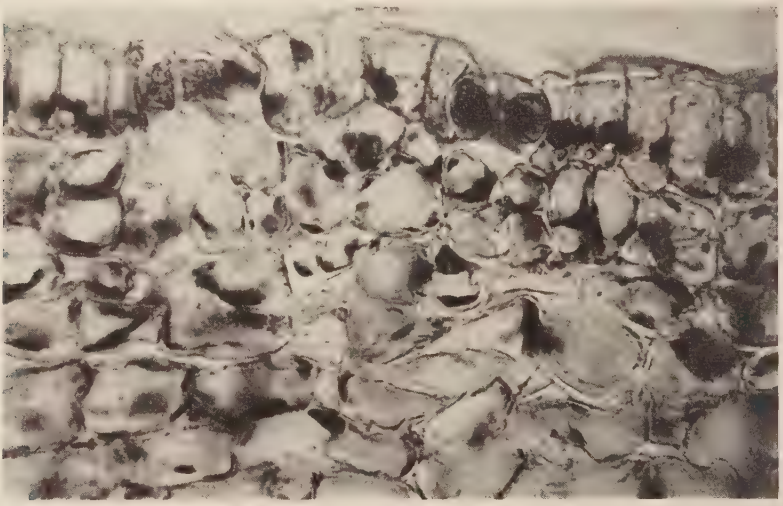


Figure 46. Similar to Figure 45 but representing another blossom and showing two stomata. Magnified 780 ×.

blossoms, though the cuticle and nectarthodes are not as prominent. While the cuticle (Figures 43 and 44) is somewhat thinner, it can be readily distinguished by comparing it with epidermal cells of the outer receptacle wall (Figures 45 and 46), and with the walls of the cells below the epidermis.

The nectarthodes are not often buried in deep pits, as so frequently occurs in pear blossoms, but are commonly found at the same level or only slightly below that of the adjoining tissue (Figures 47 and 48). They are seemingly not as abundant as in pear blossoms, though this has not been definitely determined.

The nectar-secreting tissue is quite comparable to that in pear blossoms. The cell walls are very thin, the intercellular spaces are small, and the protoplasts often stain heavily (Figure 47), which is suggestive of highly active secretory tissue.

When fully open apple blossoms are sprayed with pure cultures of *Er. amylovora*, penetrations occur far more often on stigmas, anthers, outer receptacle walls, and calyx lobes than on

the nectarial surface, and unless the spraying or atomizing is done with considerable force, penetrations of the nectarial surface are likely to be few or none at all. However, studies made of such penetrations reveal essentially the same things as in pear. The bacteria penetrate only through the openings made by the guard cells of the nectarthodes; and within 48 to 52 hours following inoculation, they may be found within the tissue below and immediately around the nectarthodes (Figures 49 and 50).

With slight modification, the pathological histology of the infected receptacle tissue of apple is comparable to that of pear blossoms. As in the latter, there is excellent evidence for the passage of bacteria not only in air spaces, but also between cells lacking air spaces and even occasionally into the protoplasts (Fig-

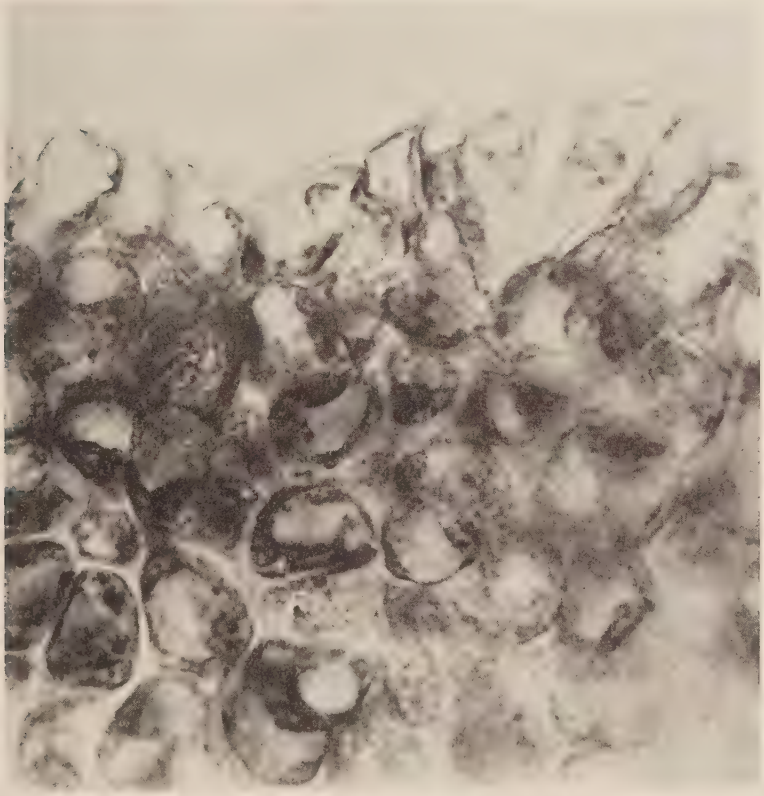


Figure 47. Angular longitudinal section of normal Jonathan receptacle in the nectarial region, showing a nectarthode in partial surface view and cuticular sculpturing seen in optical alignment on the third cell to the left of the nectarthode. Magnified 1065 ×.

Figure 48. Angular surface view of normal Jonathan receptacle in the nectarial region showing two nectarthodes. Magnified 1270 ×.

ures 51, 52, and 53). Often so much of the middle lamellae is lost within a heavily-infected region and the protoplasts are so shrunken that the outlines of the cells become very irregular and distorted. (Compare Figure 54 with 55.) Not only the cytoplasm, but the nuclei as well, show various degrees of collapse and disorganization. Within 52 hours after the inoculum is applied, the nuclei within infected tissues vary from no visible pathological change to one in which they are represented by mere fragments or collapsed and coagulated hulls. First, the outer membrane loses definition and becomes more or less ill-defined and irregular (Fig-

ure 56). The nucleolus gradually disappears, and the once oval or spherical nuclear figure shrinks to a small, undifferentiated, densely-staining, irregular mass.

The penetration by *Er. amylovora* of outer or side walls of the apple receptacle and of calyx lobes is very similar to that in the

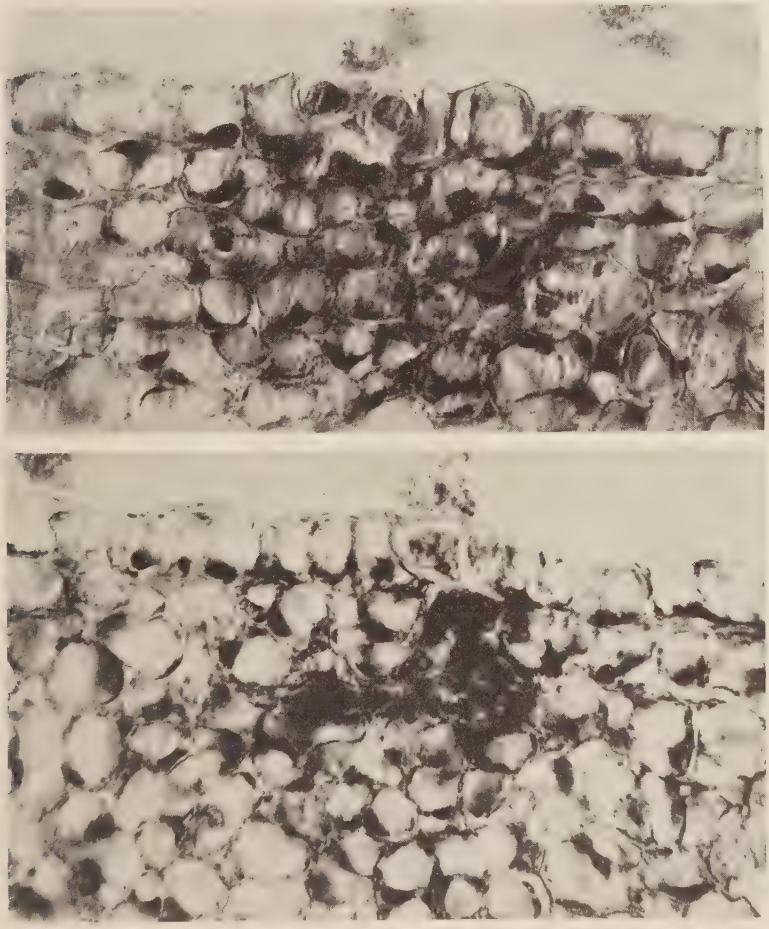


Figure 49. (Above.) Bacterial penetration through a Jonathan nectarthode and into considerable tissue beneath and around the sub-nectarthodal chamber, representing material killed 52 hours after inoculation. Magnified 775 ×. The apparent absence of bacteria in the chamber is probably an artifact induced by sectioning.

Figure 50. (Below.) Similar to Figure 49 and showing the intense discoloration of protoplasts in the invaded region below a nectarthode 52 hours after inoculation. Magnified 775 ×.

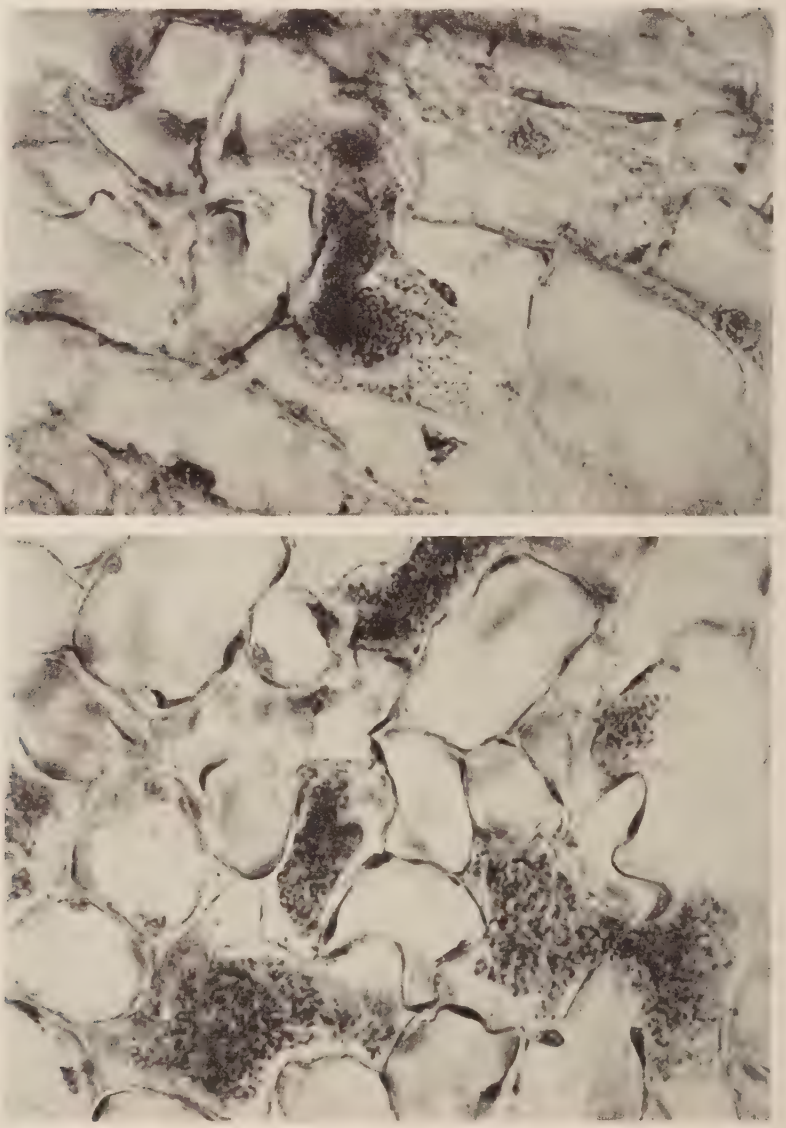


Figure 51. (Above.) Jonathan receptacle tissue, 52 hours after inoculation, showing the intracellular passage of bacteria. Magnified 950 ×.

Figure 52. (Below.) Jonathan receptacle, 52 hours after inoculation, showing the inter- and intracellular passage of bacteria deep in the tissue. Most of the bacterial masses clearly observable are located within cells and not intercellular spaces. The intercellular spaces are small and at least five, non-occupied, are to be seen in this view. Magnified 950 ×.

Figure 53. (Above.) Jonathan receptacle, 52 hours after inoculation, showing extensive bacterial invasion, mainly between cells. Note the marked loss of cellular turgidity as shown by the wrinkled and curved walls. Magnified 780 ×.

Figure 54. (Below.) Jonathan receptacle, 52 hours after inoculation, showing extreme plasmolysis and coagulation of host protoplasts, and the flaccid nature of the cell walls. Compare with Figure 55. Magnified 580 ×.

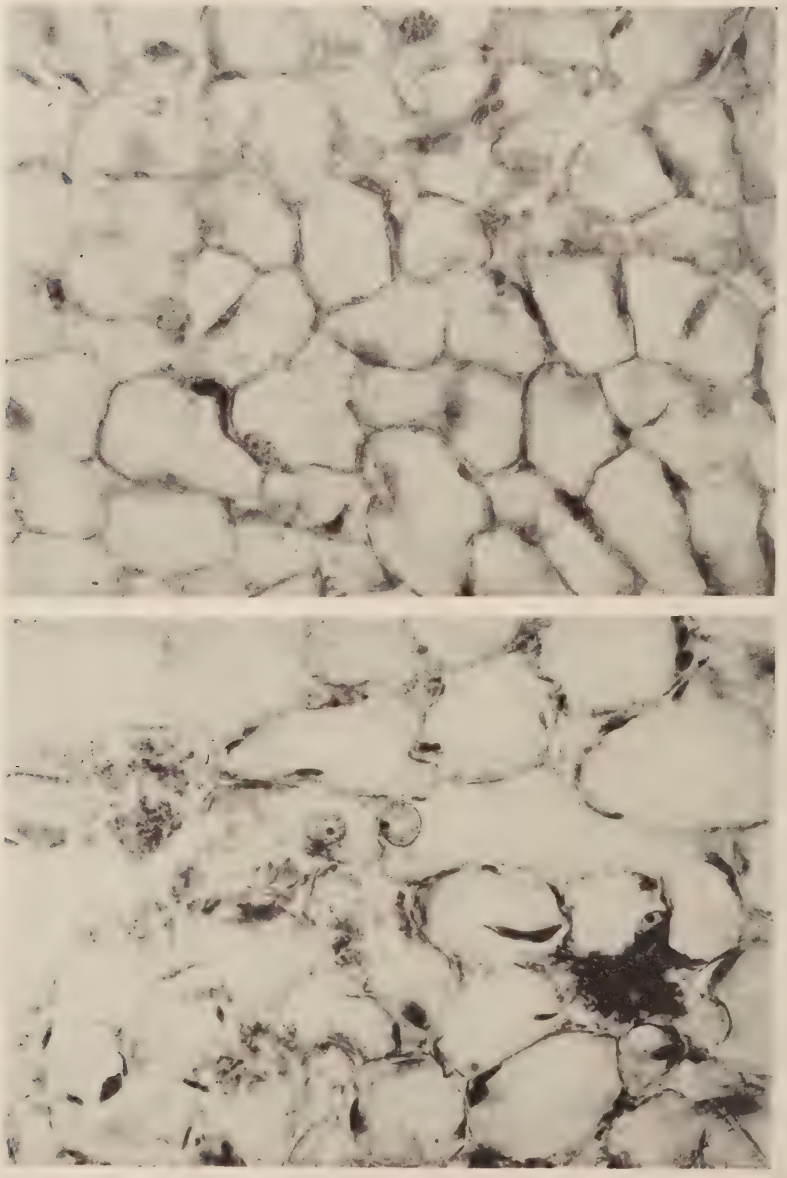


Figure 55. (Above.) Approximately the same receptacle region shown in Figure 54, but representing an infection in which the disease has not progressed as far. Note the turgid and regular outline of cell walls. Magnified 780 ×.

Figure 56. (Below.) Loss of definition and collapse of host nuclei in infected Jonathan receptacle tissue, 52 hours after inoculation. Note the partial collapse of the host nucleus in the cell to the left of center, and the more normal appearance (in killed and stained preparations) of the nucleus to the right. Magnified 580 ×.

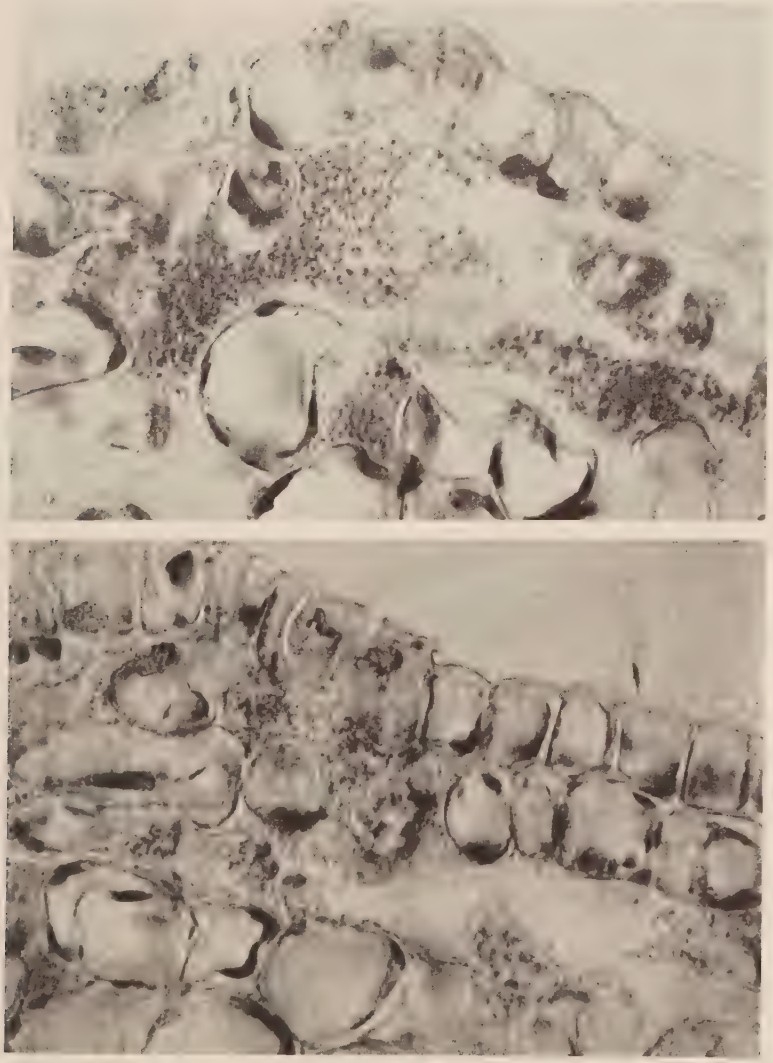


Figure 57. (Above.) Artificial infection on Jonathan calyx lobe showing bacterial invasion of part of the sub-stomatal chamber and adjoining tissue, 52 hours after inoculation. Is there evidence of dissolution of cells in this view? Note the cell toward the center which is immediately below the epidermis and how it shows attachment in this plane only to the epidermal cell. Also note the abnormality of cells below the sub-stomatal cavity, particularly the convexity of upper cell walls of two of the cells, and compare with Figure 58. Magnified 1040 ×.

Figure 58. (Below.) Jonathan calyx lobe, 52 hours after spray-inoculation, showing the presence of bacteria in the sub-stomatal chamber and in adjoining tissue. A less advanced stage of disease than that shown in Figure 57. Magnified 1040 ×.

nectarial region (Figures 57 and 58), the bacteria penetrating through the stomata as Miller *(7)* has previously shown. Within a given number of hours, such penetrations appear fully as rapid as that in apple nectarial tissue, and the progressive invasion of the tissue is apparently just as great as in the latter. However, the difficulties of getting good paraffin sections of apple receptacle tissues have hindered a clear understanding of any difference that

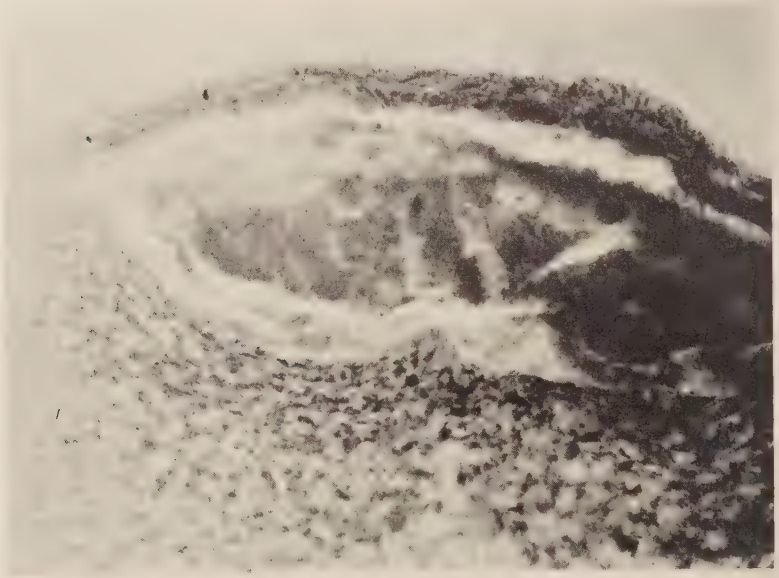


Figure 59. Huge bacterial pocket in Jonathan receptacle tissue in which the invasion has become localized in a region about five layers of cells below the nectarial surface. See text for further details. Magnified 352 ×.

may be expected in a given length of time in the degree of invasion through stomata when compared to nectarthodes.

Unlike the type of receptacle invasion to be found in most apple blossoms subjected to artificial infections, in which the bacteria penetrate deeply into the tissues within 2 to 3 days after inoculation, another and apparently less frequent type has been found. This consists of a much more restricted invasion, in which the bacteria become localized in large-sized cavities several layers beneath the upper receptacle surface (Figure 59). Such cavities filled with bacteria and with little or no additional invasion of deeper tissues, have been noted in blossoms inoculated 7 days previous to being killed and fixed for microtome sectioning. The cavities result from a localized disintegration of several layers of

cells and also from a mechanical tension around the center of bacterial growth. The tension causes both a pushing upward of the tissue above the mass of bacteria, and a downward push which makes the lower tissue curve or fold in a plane below their normal level. Likewise, there is a suggestion of a wound response and a suberization of several layers of cells immediately below the bacteria-filled cavity. Is such a localized invasion characteristic of infected apple or pear blossoms which abscise instead of hanging on to the tree for relatively long periods of time? It is shown

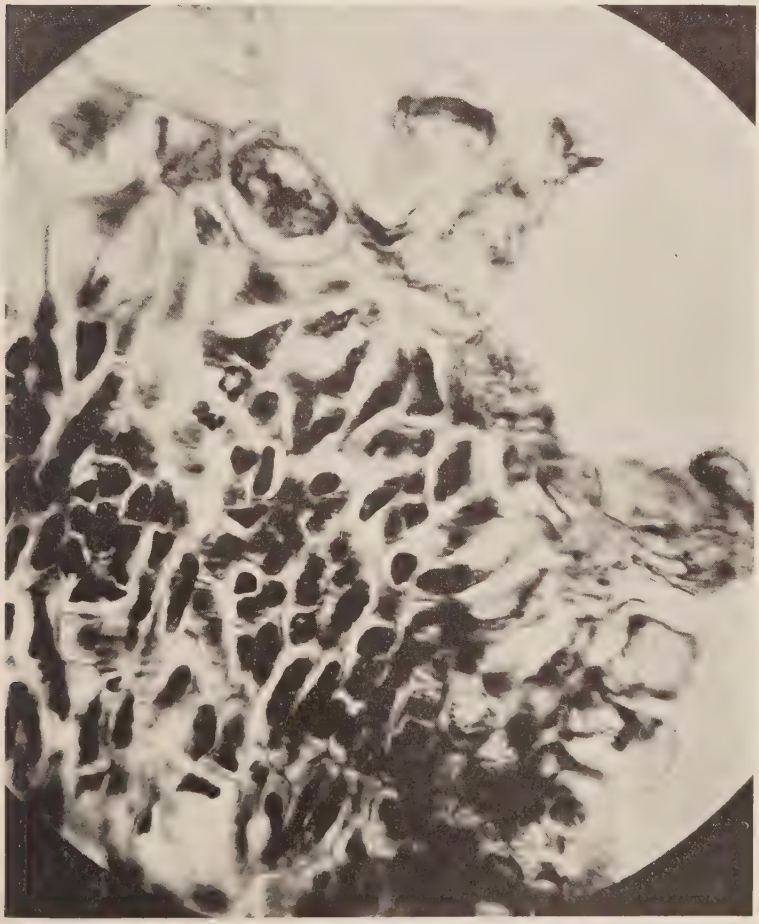


Figure 60. Longitudinal section of normal Jonathan stigma showing large, thin-walled outer cells, the papillae. Magnified 640 ×.

in Arkansas Experiment Station Bulletin 330 that in some seasons abscission of apple and pear blossoms infected with *Er. amylovora* is a rather common occurrence.

One of the most common means of entrance of *Er. amylovora* into apple blossoms in artificial spray-inoculations is through stigmatic surfaces. It is, of course, well known that these surfaces are secretory or glandular in nature, and exude a liquid which

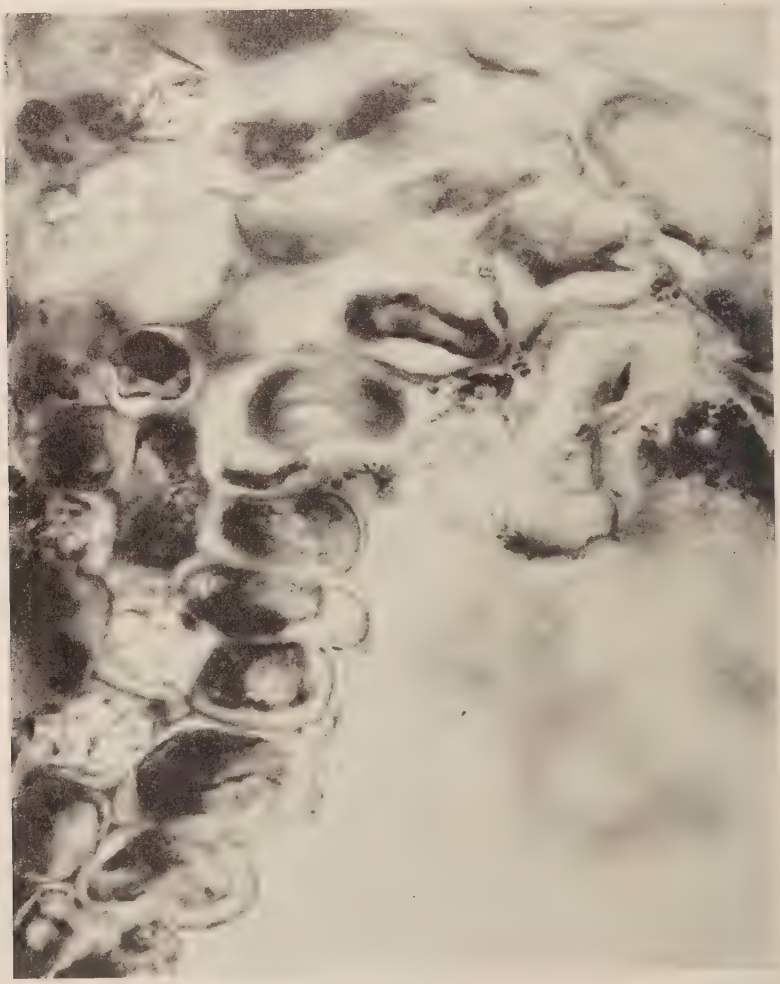


Figure 61. Longitudinal section of Jonathan stigma (upper right) and style showing the lack of cuticle on the surface stigmatic cells compared with stylar cells. Magnified 1250 ×. Material killed 23 hours after inoculation.

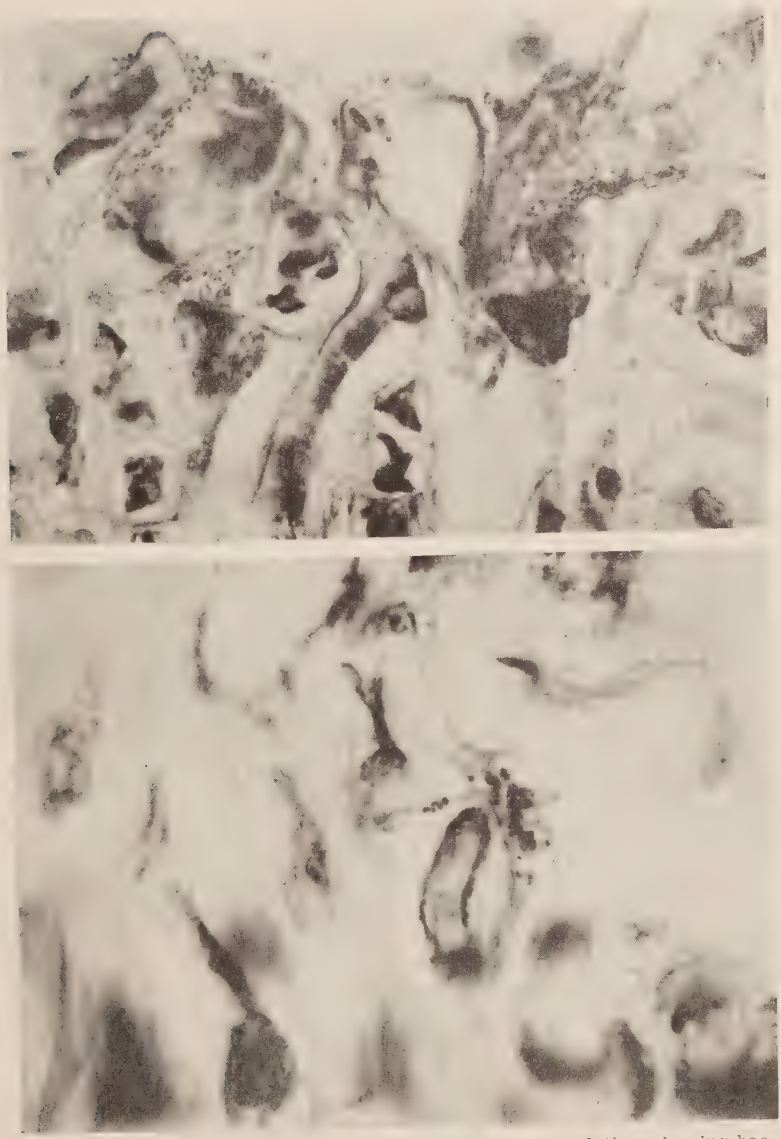


Figure 62. (Above.) Jonathan stigma, 23 hours after inoculation, showing bacterial invasion of tissue by passage between two adjoining cells, upper left. Magnified 950 ×.

Figure 63. (Below.) Stigmatic invasion within 23 hours after inoculation. Note the presence of a group of bacteria between two adjoining cells within the stigmatic tissue, toward the center, and close to the surface cells. Magnified 1425 ×.

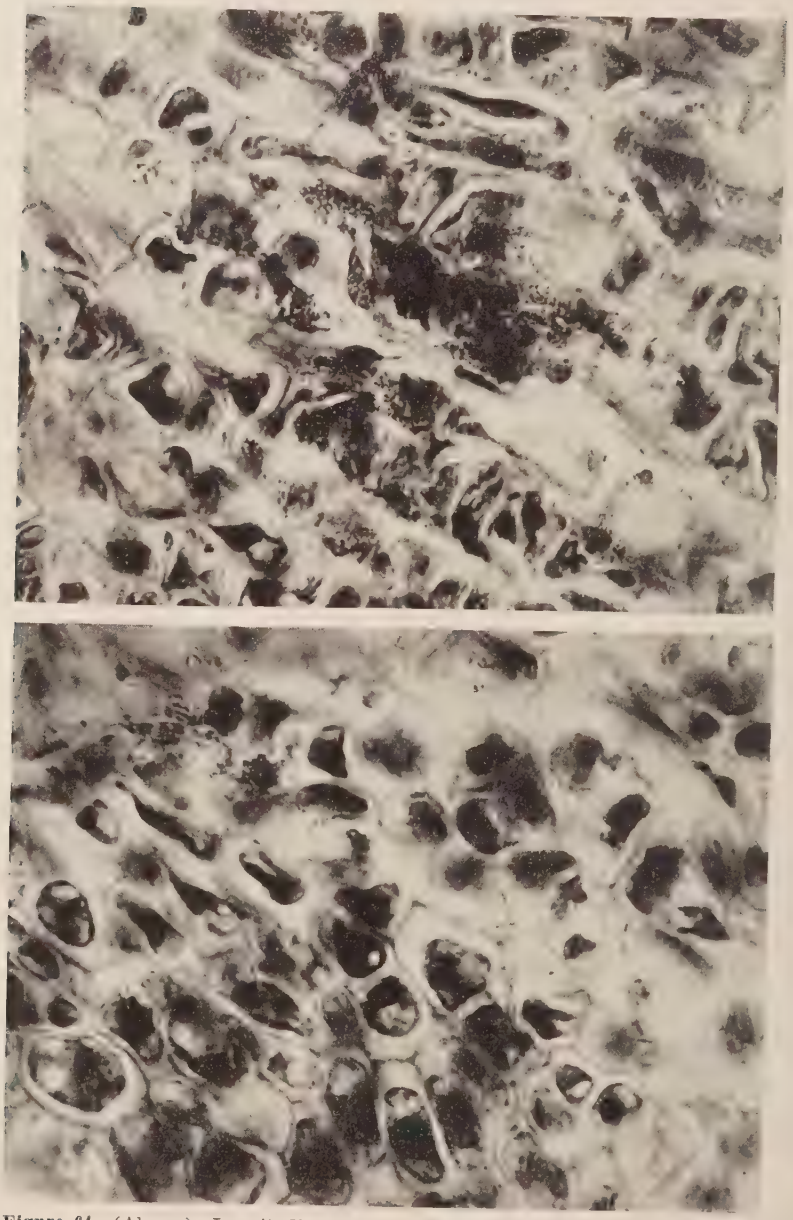


Figure 64. (Above.) Longitudinal section of Jonathan stigma and style, 23 hours after stigmatic inoculation, showing the nature and depth of bacterial penetration. Magnified 950 ×.

Figure 65. (Below.) Representing similar material to that shown in Figure 64 with the bacterial invasion further advanced within the tissue in the same time interval. Magnified 950 ×. (Stigmatic tissue is shown at right.)

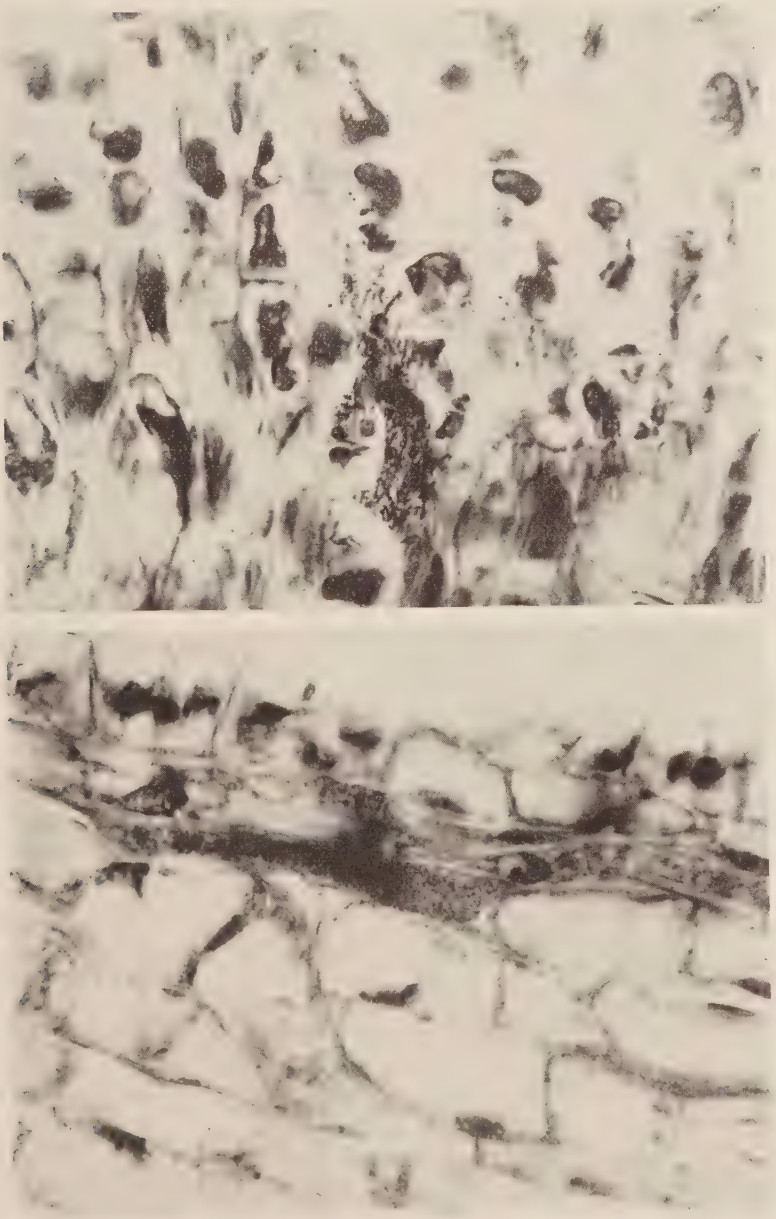


Figure 66. (Above.) The bacterial strands reaching into the style within 23 hours after stigmatic inoculation, and migrating between the cells. Magnified 950 ×.

Figure 67. (Below.) The migration of bacterial strands in style, close to the base, within 52 hours after inoculation. Magnified 950 ×.

is conducive to the adherence of pollen grains to the stigma and also conducive to their germination. In normal apple blossoms, the outermost layer of cells consists of large bladder-like structures with thin walls, apparently devoid of cuticle, that more or less sparsely stud the surface (Figure 60). They constitute the papillae observed at low magnifications. The area between these projecting cells is made of similarly large thin-walled cells. The

Figure 68. Compact bacterial masses migrating in the stylar tissue, two to six layers of cells beneath the epidermis. Magnified 450 ×.

projecting cells are so delicate that they are readily broken or dismembered. Beneath this outermost layer, the two, and occasionally three, succeeding layers are likewise made up of relatively large thin-walled cells with ample intercellular spaces between them. The cells which lie still deeper are smaller and possess dense protoplasts which stain very heavily and which show considerable plasmolysis when killed with either formalin-acetic acid-alcohol mixture or chromo-acetic acid. When the outer cell walls of the stigma are compared with those of the style (Figure 61), there appears to be no question about the lack of cuticle on the former. The stylar epidermal cells show a rather thick outer layer, which is refractory to the stains previously described, and is quite similar to that found on outer receptacle walls. The outer stigmatic cells, on the other hand, show no such thick, glistening layer.

Within 24 hours after stigmatic surfaces of Jonathan blossoms are inoculated by brushing them gently with a fine camel's hair brush containing a water-suspension of *Er. amylovora*, the bacteria may be seen variously disposed on the surface of the

stigmatic tissue and for various depths within the tissue itself (Figures 62 and 63). The bacteria may be traced from the surface of the large outer cells into the underlying tissue, the entrance being gained apparently by an intercellular space or by a dissolution of the thin outer walls, which allows the passage of the bacteria between two adjoining outer cells (Figure 62). Having gained entrance into the interior, they spread with considerable rapidity, and have been traced in the 24-hour period following

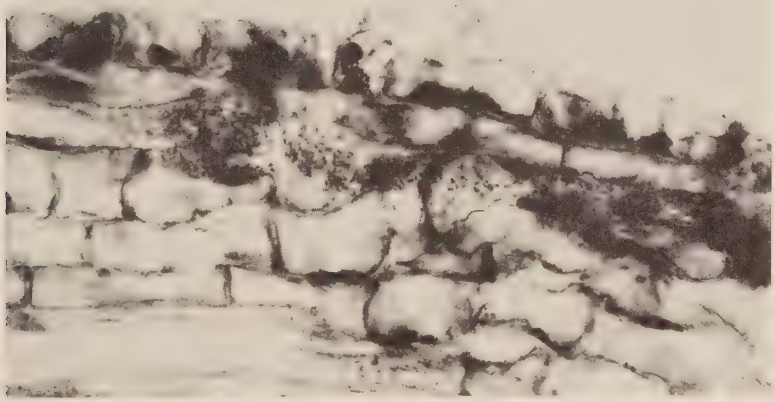


Figure 69. The arrival of bacterial strands in the region where the style joins the receptacle within 52 hours after stigmatic inoculation. Magnified 570 ×.

inoculation as deep as 10 layers of cells below the point of entrance (Figures 64 and 65). The chief mode of penetration is by means of intercellular spaces (Figure 66), though there is indication that the passage is enhanced by localized dissolution of middle lamellae and the wedging of bacterial strands between cell walls that had been joined together previously.

Fifty-two hours after inoculation of stigmatic surfaces, the bacterial strands have been traced downward through the entire length of the style and even in the upper part of the receptacle. As they make their way downward within the style, they may be seen in very heavy or light masses within intercellular spaces or in manufactured spaces between those cells that are two to six layers beneath the epidermis (Figures 67, 68, and 69). The fact that they may often be seen in long continuous strands at one optical level clearly is suggestive of their ability to dissolve walls in stylar tissues. No such long intercellular spaces are to be found in normal styles. The dissolution of walls in this instance is more or less confined to those walls that are perpendicular to the

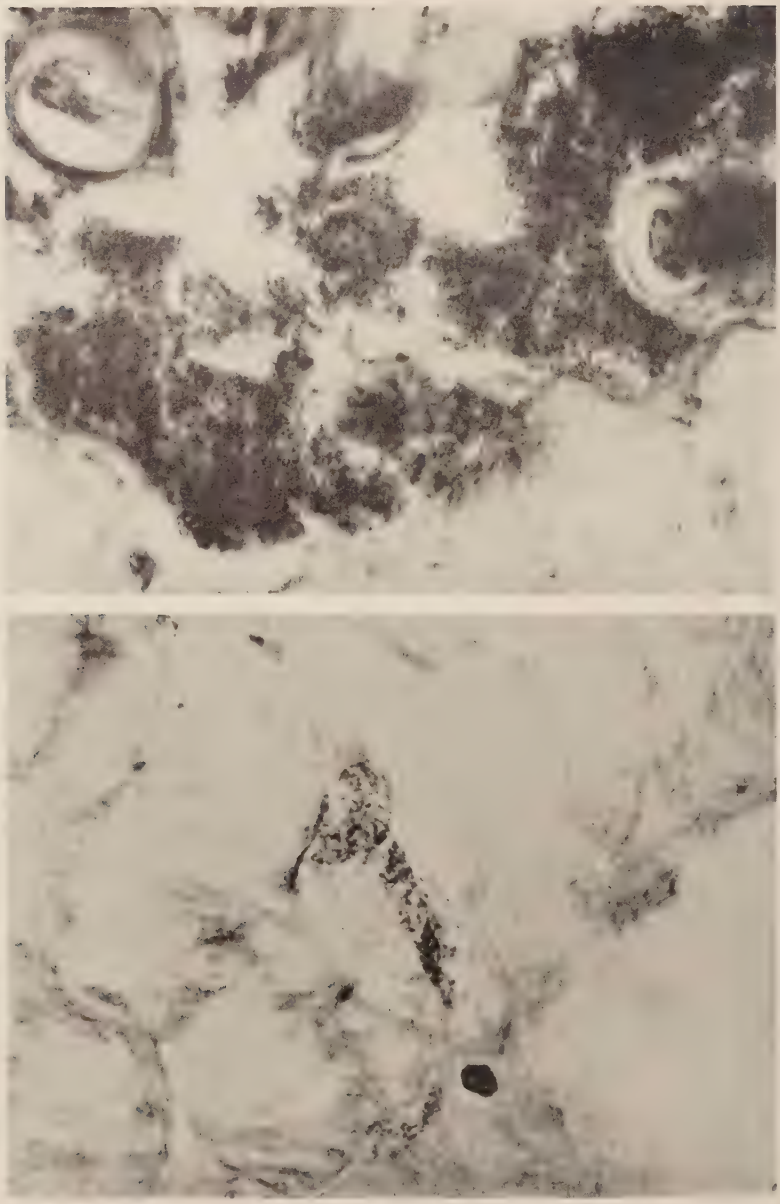
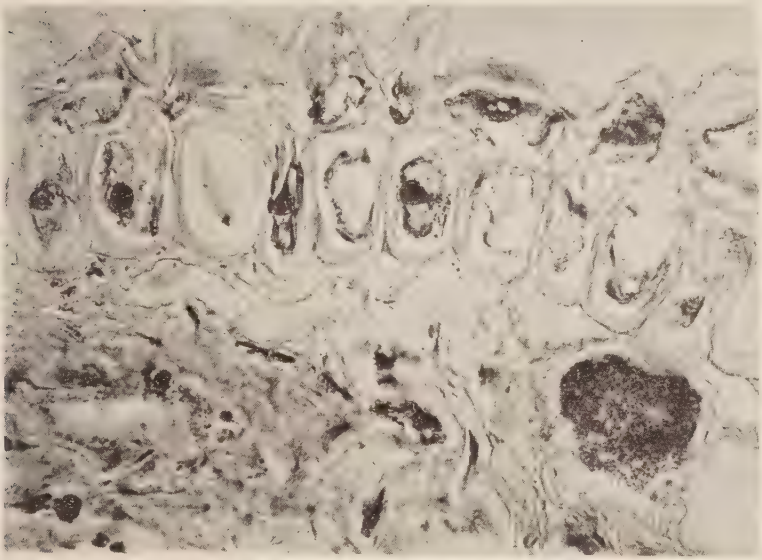


Figure 70. (Above.) Jonathan anther locule, 52 hours after inoculation, showing the dense masses of bacteria occupying the locule and indicating a rapid multiplication of bacteria. Magnified 950 ×.

Figure 71. (Below.) The migration of a bacterial strand in a Jonathan anther from the locule into the adjoining tissue within 24 hours after inoculation. Anther locule represented at the lower right. Magnified 1756 ×.



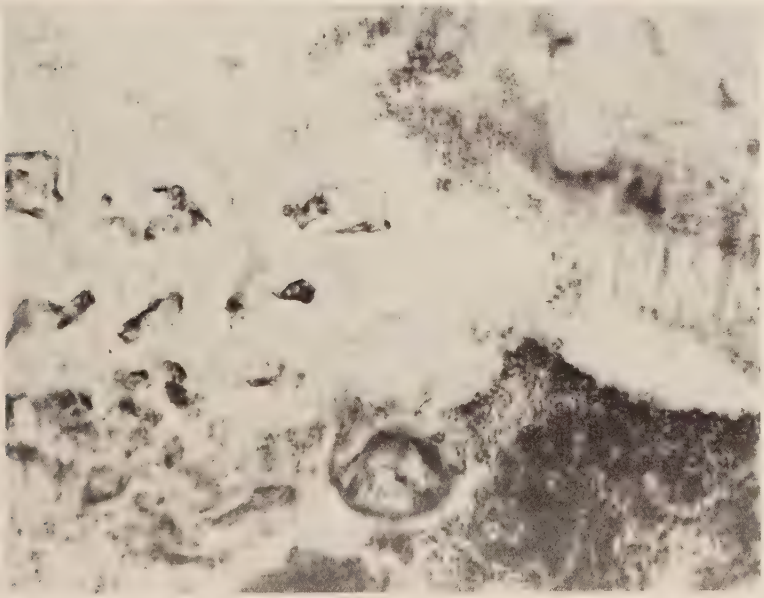


Figure 72. (Above.) Dense mass of bacteria occupying the disorganized tissue bordering the anther locule. Magnified 665 ×.

Figure 73. (Below.) Partly open anther, upper part, showing the occupation of bacteria reaching from the opening into the locule, and the occupation of the locule. Magnified 950 ×.

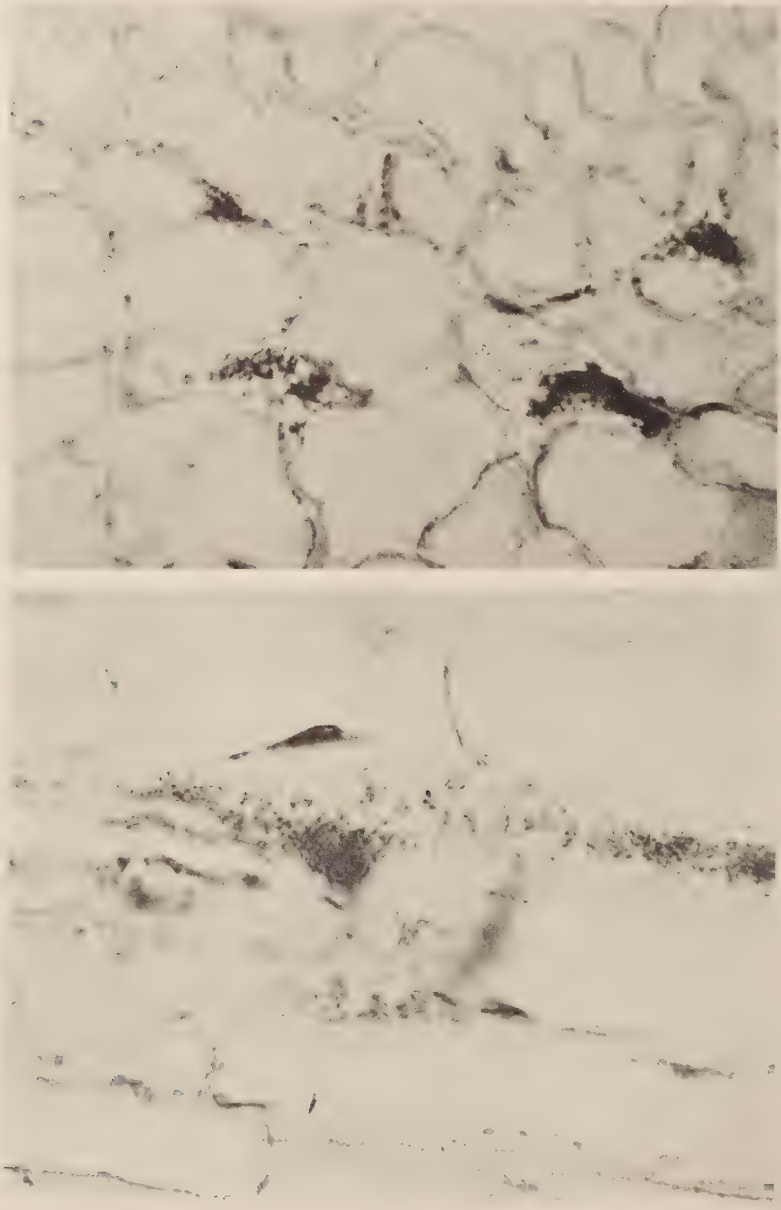


Figure 74. (Above.) The migration of bacteria within anther tissue reaching the endothecial layer, left of center, within 24 hours after inoculation. Magnified 920 ×.

Figure 75. (Below.) Bacterial invasion of filament 52 hours after inoculation of anther. Magnified 950 ×.

long axis of the style; and the evidence suggests not only a dissolution of middle lamellae but also of walls that have undergone some degree of differentiation and possibly a deposition of cellulose. In these studies of progressive invasion, the evidence indicates that bacteria invade more and more tissue as they continue to grow and multiply. There is no evidence to indicate that their progress is the result of the movement of a mass of bacteria from one space to another with an emptying of the space originally occupied. The same sort of progressive invasion seems to hold true whether the avenue of entrance is a nectarthode, a stoma, or a stigmatic surface, although in the process of killing, sectioning, and staining, the bacteria are often washed out from some of the tissue so that they cannot always be traced satisfactorily.

Another type of apple blossom tissue which serves as an excellent nidus or path for invasion of *Er. amylovora* is to be found in anthers. When partly open anthers are inoculated by means of smears made with a fine brush carrying a water suspension of bacteria, and the blossoms are covered with glassene bags for 24 to 48 hours, the organisms may be seen in great numbers occupying the locules and more or less completely filling them within 48 to 52 hours (Figure 70). Indeed, within 24 hours after inoculation, the bacteria may be found in great numbers not only within the locules but wending their way between the loose, thin-walled, partly-disrupted and collapsed cells, which constitute the extreme inner wall of the locule (Figure 71). Shortly after the dehiscence of the anther, these cells ordinarily collapse, and appear as considerably wrinkled or crumpled structures with protoplasts variously disorganized. The studies indicate that these dead cells serve as an excellent medium for the rapid multiplication of fire-blight bacteria. As the bacteria multiply, they are often to be found grouped in dense masses (Figures 72 and 73). In this loose, disorganized tissue, they soon come in contact either with living endothecial cells (Figure 74), or with those constituting the connective which joins the anther with the filament, or with the large mass of thin-walled cells possessing annular thickenings.

Having reached the connecting tissue between anther and filament, the bacteria pass down the latter, and within 52 hours after inoculation they may be readily observed in masses between the cells (Figure 75), much in the same manner and in similar regions as described for styles. The length of time taken to reach the receptacle by the bacterial masses progressing downward in the filaments has not been determined. In a number of blossoms that had received anther inoculations, the receptacles showed large numbers of bacterial strands 7 days after being inoculated, but as the material, representing 3- to 6-day intervals after inoculation, was badly torn in the process of sectioning, the extent of invasion

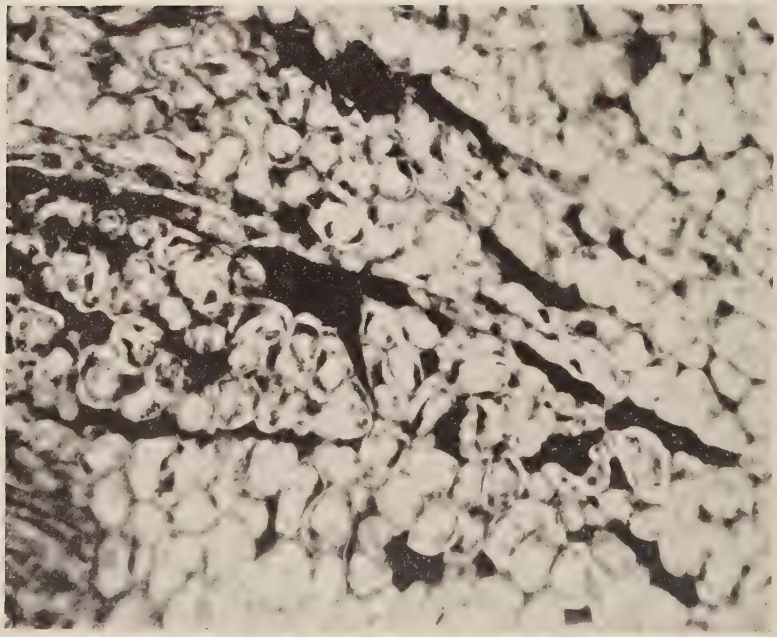


Figure 76. (Above.) Bacterial occupation of receptacle tissue 7 days after inoculation of anthers. Magnified 920 ×.

Figure 77. (Below.) The extreme density of bacterial strands occupying the receptacle 7 days after inoculation of anthers. Magnified 352 ×.

in these intervals remains to be investigated. However, at the end of 7 days after anther inoculation, the extent of bacterial invasion of the receptacle is so marked (Figures 76 and 77) that there can be little doubt of the receptivity of anthers to infection and their ability to act at times as channels of invasion for the receptacle.

Summary and Conclusions

Lack of knowledge of floral penetration by the fire-blight pathogen and of the normal structure of nectarial regions of apple and pear blossoms, which hindered a proper understanding of the floral parts requiring protection against invasion by *Erwinia amylovora* (*Bacillus amylovorus*), indicated the need for further investigation of these subjects. The studies here reported reveal the following:

The nectarial region of pear blossoms, unlike that of apple blossoms, resides in an open, shallow, fully-exposed, saucer-shaped tissue, located between the point of emergence of the styles and the stamens, the latter marking the periphery of the nectar-exuding tissue.

In pear blossoms the droplets of nectar are excreted through stoma-like structures, termed "nectarthodes," which often occur at the base of deep pits or depressions in the nectarial surface. These nectarthodes are made up of two guard cells which closely resemble those of ordinary stomata and the flow of nectar appears to be regulated in the same manner as the flow of air or water vapor through stomata.

There is a well-defined cuticle covering the whole of the nectarial surface. It is fully as thick as that lining the outer or side walls of the receptacle, and as protective in preventing the entrance of bacteria. The only openings free from cuticular covering that may serve as avenues of bacterial invasion of nectarial tissue are those made by the nectarthodes.

Beneath the epidermal cells lining the nectarial region, there is a zone of tissue 12 to 15 layers of cells deep, which probably functions in the manufacture of nectar. This tissue is composed of relatively small, compact cells which stain heavily with cytoplasmic stains, and possess very thin cell walls which do not respond well to cellulose stains. This tissue forms a very receptive region for the growth and multiplication of *Er. amylovora*.

Within 24 to 48 hours following inoculation of open, nectar-secreting pear blossoms with pure cultures of the fire-blight pathogen, the bacteria may be found in great numbers in localized areas on the surface of the nectarial tissue, and also penetrating to various depths into the tissue. The heavy, localized surface growth of bacteria corresponds in general to the areas occupied

by droplets of nectar, as in the nectar-filled pits above the nectarthodes, and clearly indicates that pear nectar forms an excellent medium for the multiplication of these bacteria.

From the masses of bacteria gathered above the nectarthodes, extensions have been traced through the openings made by the guard cells which reach into the interior chambers below the nectarthodes. These bacterial extensions reaching into the tissues from surface growth, are considered to be an expression of the multiplication of bacteria in the nectarial medium which is continuous at times from the embedded sub-nectarthode chamber to the exposed surface of nectarial tissues.

The entrance of *Er. amylovora* into nectarthodes is, therefore, considered to be in the nature of saprophytic growth, wending its way through a natural opening into the interior.

The extension of bacterial strands from the sub-nectarthode chambers has been traced through as many as eight layers of cells below or to the sides of the chambers in a period of 48 hours after the blossoms were inoculated. The progressive invasion is accompanied by discoloration, plasmolysis, coagulation, and disintegration of the protoplasts of cells adjoining or in the immediate vicinity of the chamber.

Tissue invasion consists not only of the well-known passage through intercellular spaces but of localized dissolution of middle lamellae and delicate cell walls, often resulting not only in the separation of adjoining cells but of actual penetration into protoplasts. Invasion of protoplasts is found in infections that are not more than 48 hours old.

Histological studies of the nectarial tissues of normal apple blossoms reveal essentially the same structures as in pear blossoms. While the cuticle and nectarthodes are not as conspicuous as in pear flowers, they are present and apparently function similarly. Likewise, the manufacturing and secreting of nectar is performed by small, very thin-walled cells much as in pear blossoms.

When fully open apple flowers are sprayed with pure cultures of *Er. amylovora,* penetrations occur more frequently on stigmas, anthers, outer receptacle walls, and calyx lobes than on the nectarial tissue. The mode of invasion through nectarial tissue is essentially the same as in pear blossoms.

The stigmas of apple pistils form especially receptive structures for the invasion of fire-blight bacteria. The outermost layer of cells of the stigmas possess no cuticle and their thin walls are readily acted upon by this pathogen. Within 24 hours after inoculation the bacteria may be traced from the surface, making their way through intercellular spaces or between two adjoining cells by an apparent dissolution of the walls, and reaching as deep as

10 layers of cells below the point of entrance. Within the stigmatic and stylar tissue, the progressive penetration is chiefly by means of intercellular spaces with indication of localized dissolution of middle lamellae and wedging of bacterial strands between cell walls that had been joined together previously.

Fifty-two hours after inoculation of apple stigmas, bacterial strands have been traced through the entire length of the style and reaching into the upper part of the receptacle.

Open apple anthers have also been found to serve as excellent avenues of invasion. Within 24 hours after inoculation, the bacteria have been found in great numbers in the locules and making their way between the loose, thin-walled, partly-collapsed cells which constitute the extreme inner wall of the locule. These cells appear to serve as a medium for the growth and multiplication of the bacteria. Making their way largely through intercellular spaces, they reach the filament by means of the connective, and within 52 hours after inoculation, they have been located within the filaments. After a passage down the length of the filaments, they have been observed in the receptacle.

These studies indicate that if blossom blight of pears is to be controlled by means of sprays possessing germicidal properties, it is very essential to cover the nectarial disks with the germicide. The nectarial surface appears to be the most vulnerable avenue for invasion of fire-blight bacteria in open blossoms, and open-blossom infections are by far the most common in pears.

In apple blossoms, the studies here reported suggest the necessity of a protective coating, particularly on stigmas and anthers rather than on nectarial surfaces, for the prevention of blossom blight, although nectarial tissues, outer receptacle walls, and calyx lobes serve at times as centers of penetration.

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